PROCEEDINGS

AND

TRANSACTIONS

OF THE

LIVERPOOL BIOLOGICAL SOCIETY.

VOL. XXXVII.

SESSION 1922-1923.

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1908—1909	Prof. B. MOORE, M.A., D.Sc.
1909—1910	R. NEWSTEAD, M.Sc., F.E.S.
1910—1911	Prof. R. NEWSTEAD, M.Sc., F.R.S.
1911—1912	J. H. O'CONNELL, L.R.C.P.
1912—1913	JAMES JOHNSTONE, D.Sc.
1913—1914	C. J. MACALISTER, M.D., F.R.C.P.
1914—1915	Prof. J. W. W. STEPHENS, M.D., D.P.H.
1915—1916	Prof. ERNEST GLYNN, M.A., M.D.
1916—1917	Prof. J. S. MACDONALD, L.R.C.P., F.R.S.
1917—1918	JOSEPH A. CLUBB, D.Sc.
1918—1919	Prof. W. RAMSDEN, M.A., D.M.
1919—1920	HUGH R. RATHBONE, M.A., J.P.
1920—1921	Prof. P. G. H. BOSWELL, O.B.E., D.Sc
1921—1922	HERBERT R. RATHBONE, B.A., C.C.

SESSION XXXVII, 1922-1923.

President :

Prof W. J. DAKIN, D.Sc., F.Z.S., F.L.S.

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ANGUS JOHNSTONE.
Prof. J. JOHNSTONE, D.Sc.
W. S. LAVEROCK, M.A., B.Sc.
Prof. McLEAN THOMPSON, D.Sc.

Representative of Students' Section :

Miss EDNA LIND, B.Sc.

REPORT OF THE COUNCIL.

DURING the Session 1922-23 there have been six ordinary evening meetings which have been well-attended. The change of time and the special effort at the beginning of the year, together with the interest aroused by the Associated Societies' Soiree in November, in which the Society took a not inconsiderable part, have resulted in an important increase of membership.

In February we received a visit from Prof. Goldschmidt, of Berlin, which was much appreciated. The subject of his lecture, which was delivered in English, was "The Determination of Sex."

The May meeting took the form of an exhibition of specimens, apparatus, etc., by the members, and the staffs of the Zoology, Geology, and Oceanography Departments, and was very successful.

The President and Council wish to thank all who rendered assistance.

Two excursions were held: in May to Bromborough, and in June to Delamere Forest.

In November, the Society received a sad blow by the unexpected death of Lady Herdman, whose loss has been greatly felt in many ways.

The Library continues to make satisfactory progress, and additional important exchanges have been arranged.

The Treasurer's statement and balance sheet are appended.

The members at present on the roll are as follows:—

Members	52
Associate Members	29
Student members, including Students' Section, about	30
TOTAL	111

SUMMARY OF PROCEEDINGS AT THE MEETINGS.

The first meeting of the thirty-seventh session was held at the University on Thursday, October 19th, 1922.

The retiring President, Herbert R. Rathbone, Esq., B.A., J.P., C.C., installed his successor, Prof. W. J. Dakin, D.Sc., F.Z.S., F.L.S., in the chair.

The new President spoke at some length on the aims and value of the Society, and then gave a lecture, illustrated by slides and diagrams, on the "Life of the Honey Bee," dealing with the subject largely from the point of heredity and sex-determination (see "Transactions," p. 1).

A cordial vote of thanks was passed, on the motion of Prof. Sir Wm. Herdman, C.B.E., D.Sc., F.R.S., seconded by Mr. R. C. Moore, M.A., M.Sc., M.Ed.

The meeting fixed for the ninth of November was abandoned owing to the sudden death of Lady Herdman.

The second meeting of the thirty-seventh session was held on December 14th, 1922, in the Botany Department. The President occupied the chair.

1. A resolution expressing the regret of the Society at the loss sustained by the death of Lady Herdman, and of sympathy with Sir William and Miss Herdman, was carried.
2. The Report of the Council on the Session 1921-22 (see "Proceedings," Vol. XXXVI, p. viii) was submitted and adopted.
3. The Treasurer's Balance Sheet for the Session 1921-22 (see "Proceedings," Vol. XXXVI. p. xvi) was submitted and approved.

4. The following Office-bearers and Council for the ensuing Session were elected :—Vice-President, Herbert R. Rathbone, C.C. ; Hon. Treasurer, W. J. Halls ; Hon. Librarian, Miss May Allen, B.A. ; Hon. Secretary, W. Rimmer Teare, A.C.P. ; Council, Mrs. Bisbee, M.Sc., Prof. P. G. H. Boswell, O.B.E., D.Sc., S. T. Burfield, B.A., M.Sc., J. A. Clubb, D.Sc., G. Ellison, Miss Alwen M. Evans, M.Sc., Miss E. C. Herdman, Prof. Sir William Herdman, C.B.E., D.Sc., F.R.S., Angus Johnstone, Prof. J. Johnstone, D.Sc., W. S. Laverock, M.A., B.Sc., Prof. McLean Thompson, D.Sc., F.L.S.
5. A lecture was given by Prof. McLean Thompson, D.Sc., on " Plant Mechanics," showing the methods adopted by plants for securing mechanical safety, and discussing how mechanical tissues had arisen.

The third meeting of the thirty-seventh session was held in the University on Thursday, January 11th, 1923, Sir Wm. Herdman in the chair.

1. Prof. J. Johnstone, D.Sc., presented the reports of the Oceanographical Department, and of the Port Erin Station (see " Transactions," p. 9).
2. Prof. Johnstone then delivered a lecture on " Vital Production," and showed how, if living organisms were to be preserved from depletion certain steps must be taken, and how such steps could be determined and directed by a study of biology.

The fourth meeting of the thirty-seventh session was held, in conjunction with the Zoology Department, on Thursday, 15th February, 1923, the President in the chair.

Prof. R. Goldschmidt, of Berlin, lectured to a large audience on "Sex-determination."

On the motion of the Vice-Chancellor, seconded by Prof. Blair Bell, the lecturer was heartily thanked for his address.

The fifth meeting of the thirty-seventh session was held in the University on Thursday, the 8th March, 1923, the President in the chair.

Prof. Sir Wm. Herdman opened a discussion on "The Darwinian Theory of Natural Selection," which, he contended, offered the most satisfactory explanation of the problem of biological development.

An animated discussion followed, in which the President, the Vice-Chancellor, Prof. McLean Thompson, Prof. Johnstone, and the Rev. Jas. Hamilton took part.

The sixth meeting of the thirty-seventh session was held on the 10th May, 1923, and took the form of a Soiree and Exhibition of objects of biological interest by the members and the staffs of the Zoology, Geology and Oceanography Departments. There was a very large attendance, and the meeting proved very successful.

Prof. R. Newstead, M.A., F.R.S., gave a short lecture on the Tsetse Fly, illustrated by slides and by specimens from the School of Tropical Medicine.

On May 26th, 1923, an excursion was held at Bromborough, led by Mr. S. J. Burfield, B.A., M.Sc., and on June 9th a visit was paid to Delamere Forest.

Prof. Dakin was elected President for 1923-24, and the Secretary was appointed delegate to the British Association meeting at Liverpool.

There was not a great attendance of members at these excursions, and the desirability of a Spring and Summer excursion programme remains to be discussed.

LIST of MEMBERS of the LIVERPOOL BIOLOGICAL SOCIETY.

SESSION 1922-1923.

A. Members.

(Life Members are marked with an asterisk.)

ELECTED.

1908 Abram, Prof. J. Hill, M.D., F.R.C.P., 74, Rodney Street, Liverpool.

1919 Adami, Dr. J. G., F.R.S., Vice-Chancellor, The University, Liverpool.

1909 *Allen, Miss May, B.A., Hon. Librarian, University, Liverpool.

1918 Baldwin, Mrs., M.Sc., Zoology Dept., University, Liverpool.

1913 Beattie, Prof. J. M., M.A., M.D., The University, Liverpool.

1915 Bisbee, Mrs., M.Sc., Zoology Dept., The University, Liverpool.

1903 Booth, Chas., Cunard Building, Liverpool.

1919 Boswell, Prof. P. G. H., O.B.E., D.Sc., The University, Liverpool.

1912 Burfield, S. T., B.A., M.Sc., Zoology Department, University, Liverpool.

1886 Caton, R., M.D., F.R.C.P., 7, Sunnyside, Prince's Park, Liverpool.

1886 Clubb, J. A., D.Sc., Free Public Museums, Liverpool.

1920 Dakin, Prof. W. J., D.Sc., F.L.S., President, The University, Liverpool.

1917 Duvall, Miss H. M., M.Sc., Zoology Department, University, Liverpool.

1910 Ellison, George, 52, Serpentine Road, Wallasey.

1918 Evans, Miss Alwen M., M.Sc., School of Tropical Medicine, University, Liverpool.

1921 Fletcher, Miss Isabel, 42, Ullet Road, Liverpool.

1922 Fry, Edward, 89, Penny Lane, Liverpool.

1902 Glynn, Prof. Ernest, M.D., F.R.C.P., 67, Rodney Street.

1886 Halls, W. J., Hon. Treasurer, 2, Townfield Road, West Kirby.

1922 Heilbron, Prof. I. M., D.S.O., D.Sc., The University, Liverpool.

1886 Herdman, Prof. Sir William, C.B.E., D.Sc., F.R.S., University, Liverpool.

1921 Herdman, Miss E. C., Croxteth Lodge, Ullet Road, Liverpool.

1912 Hobhouse, J. R., 19, Ullet Road, Liverpool.

1902 Holt, Dr. A., Rocklands, Thornton Hough, Cheshire.

1903 Holt, Richard D., India Buildings, Liverpool.

1920 Johnstone, Angus, 63, Church Road, St. Michael's, Liverpool.

1898 Johnstone, Prof. James, D.Sc., University, Liverpool.

1918 Jones, Philip, Brantwood, St. Domingo Grove, Liverpool.

1896 Laverock, W. S., M.A., B.Sc., Free Public Museums, Liverpool.

1915 Macdonald, Prof. J. S., B.A., F.R.S., The University, Liverpool.

1922 McDonald, Dr. Archie W., L.R.C.P., L.R.C.S. (Edin.), Glencoe, Huyton.

1917 Milton, J. H., F.G.S., Merchant Taylors' School, Great Crosby.

1904 Newstead, Prof. R., M.Sc., F.R.S., University, Liverpool.

1913 Pallis, Mark, Tätoi, Aigburth Drive, Liverpool.

1915 Prof. W. Ramsden, M.A., D.M., University, Liverpool.

1921 Rathbone, Herbert R., C.C., Vice-President, 35, Ullet Road, Liverpool.

1903 Rathbone, Hugh R., M.A., J.P., Greenbank, Liverpool.

1890 *Rathbone, Miss May, High House, Leaves Green, Keston, Kent.

1894 Scott, Andrew, A.L.S., Piel, Barrow-in-Furness.

1908 Share-Jones, J., D.Sc., F.R.C.V.S., University, Liverpool.

1886 Smith, Andrew T., Solna, Croxteth Drive, Liverpool.

1920 Southwell, T., M.Sc., School of Tropical Medicine, University, Liverpool.

1903 Stapledon, W. C., Annery, Caldy, West Kirby.

1913 Stephens, Prof. J. W. W., M.D., University, Liverpool.

1915 Teare, W. Rimmer, A.C.P., Hon. Secretary, 12, Bentley Road, Birkenhead.

1903 Thomas, Dr. Thelwall, 84, Rodney Street, Liverpool.

1905 Thompson, Edwin, Woodlands, 13, Fulwood Park, Liverpool.

1921 Thompson, Prof. McLean, D.Sc., The University, Liverpool.

1889 Thornely, Miss L. R., Hawkshead, Ambleside.

1888 Toll, J. M., 49, Newsham Drive, Liverpool.

1920 Walker, Prof. C., D.Sc., M.R.C.S., The University, Liverpool.

1920 Yorke, Prof. Warrington, M.D., School of Tropical Medicine, University, Liverpool.

B. Associate Members.

1916 Atkin, Miss D., High School for Girls, Aigburth Vale, Liverpool.

1922 Braithwaite, W. G., 10, Plimsoll Street, Liverpool, E.

1922 Chenery, M. B., 68, Belvidere Road, Liscard, Wallasey.

1922 Clarke, J., Varna, Hawthorn Road, Moreton, Cheshire.

1914 Cutmore, J. W., Free Public Museums, Liverpool.

1922 Evans, C. E., 84, Boswell Street, Liverpool.

1922 Fell, W., Willaston, Birkenhead.

1922 Fordham, Miss M. G. C., B.Sc., Zoology Department, The University, Liverpool.

1922 Garnett, Theodore, M.A., South Bank, Grassendale.

1916 Gleave, Miss E. L., M.Sc., Oulton Secondary School, Clarence Street, Liverpool.

1922 Hayward, Miss, 30, Belvidere Road, Liverpool.

1922 Johnson, W., Highfield, Cliff Road, Wallasey.

1922 Jones, T. A., 27, Rockfield Road, Anfield.

1920 Kewley, Miss Helen C., 10, Park Road N., Birkenhead.

1922 Legge, Miss C. M., 3, Grove Park, Liverpool.

1922 Lewis, Miss M., B.A., Oceanography Dept., University, Liverpool.

1919 Mayne, Miss C., B.Sc., 17, Laburnum Road, Fairfield.

1922 Moore, R. C., M.A., M.Sc., M.Ed., A.I.C., 25, Galloway Road, Waterloo.

1922 Mounsey, Miss G.. St. Margaret's Hostel, Princes Road, Liverpool.

1922 Muskett, T., 101, Gwladys Street, Walton, Liverpool.

1922 Porter, Charles, 10, Wellington Terrace, Liverpool.

1922 Potts, W. H., B.A., Dept. of Entomology, The University, Liverpool.

1922 Roberts, John E., 17, Darley Drive, West Derby.

1917 Swift, Miss F., B.Sc., Queen Mary High School, Anfield.

1922 Waller, J. C., B.A., Botany Dept., The University, Liverpool.

1922 Warhurst, Miss E., L.L.A., 3, Argyle Road, Anfield.

1922 Williams, Collingwood, B.Sc., Lynton, Cressington Park.

1922 Williams, Miss E., 31, Egerton Road, Wavertree.

1912 Wilson, Mrs. Gordon, High School for Girls, Aigburth Vale, Liverpool.

C. UNIVERSITY STUDENTS' SECTION.

President : Miss Edna Lind, B.Sc.

Secretary : Miss E. Lowe, B.Sc.

(Contains about 30 members.)

D. HONORARY MEMBERS.

S.A.S., Albert I., Prince de Monaco, 10, Avenue du Trocadéro, Paris.

Bornet, Dr. Edouard, Quai de la Tournelle 27, Paris.

Fritsch, Prof. Anton, Museum, Prague, Bohemia.

Hanitsch, R., Ph.D., Oxford.

THE LIVERPOOL BIOLOGICAL SOCIETY.

Cr.				In Account with W. J. HALLS, Hon. Treasurer.			Dr.
1922, Oct. 1st to Sept. 30th, 1923.	£	s.	d.	1922, Oct. 1st to Sept. 30th, 1923.	£	s.	d.
By Balance from last Session	7	16	0	To Library Insurance	2	4	0
,, Subscriptions	25	4	0	,, Advertisements in 'Post' and 'Courier'	1	19	0
,, ,, (Arrears)	2	2	0	,, Postages of Volumes	4	8	6
,, ,, Paid into Bank	1	1	0	,, Teas	4	3	3
,, ,, Associate Members	6	7	6	,, Hon. Secretary's Expenses	2	9	0
,, Refunded from Associated Soirée	1	0	0	,, Hon. Treasurer's Expenses	0	3	6
,, Sale of Volumes	14	13	9	,, Messrs. Tinling & Co. on Account	45	0	0
,, Interest on Investment	5	13	6	,, Balance in Bank	3	5	7
,, ,, from Bank	0	8	11	,, Cash in hand	0	13	10
	£64	6	8		£64	6	8

Investment—

£113 11s. 1d. War Loan 5% 1929-47 Registered

Balance owing Messrs. Tinling & Co. £13 17 10

Audited and found correct,

STANLEY T. BURFIELD

Liverpool, *October* 11*th*, 1923.

PRESIDENTIAL ADDRESS

By WILLIAM J. DAKIN, D.Sc., F.Z.S.,
Professor of Zoology, University of Liverpool.

[ABSTRACT]

THE PROBLEM OF SEX DETERMINATION WITH SPECIAL REFERENCE TO THE HONEY BEE.

IT is probably correct to state that, for Zoologists, the problem of sex determination has been solved by recent work on the chromosomes. It is true that some writers have safeguarded their views, and thereby indicated the urgent need for further research, by suggesting that the chromosome constitution of a mature or fertilised egg is itself only an expression of some underlying primary cause—perhaps, a condition of metabolism. This may be correct in some cases, but there is much evidence to show that the unfolding of one or the other type of sex in the course of development is primarily due to the presence of certain special chromosomes—the sex chromosomes (or X chromosomes) and the factors embodied within them. This does not imply that the differences between the sexes are not due to differences in metabolism. It is more than likely that the sex factors within the chromosomes direct the development by favouring and controlling certain types of metabolism. In other words the factorial constitution is generally the "horse" and the metabolism is the "cart." An example which very clearly supports this point of view is supplied by the Nematode worm, *Ancyracanthus*. The male possesses 11 chromosomes in every cell, the female has 12. Each cell giving rise to spermatozoa in the male produces four sperm cells, two of which have five chromosomes and two have six. There is no reason to believe that there is any difference between these cells (produced side by side) which would cause

the odd chromosome to go into one rather than the other, and the odd chromosome is the sex chromosome.

The eggs are all the same, and each contains the same number of chromosomes—six. It has been clearly demonstrated that if a sperm with six chromosomes fertilises an egg with six, a female results; whilst if an egg is fertilised by a sperm with five chromosomes, a male results. The sex is evidently decided by the presence of one or of two special chromosomes.

The most startling demonstration of the influence of the sex chromosomes is, however, that discovered by Bridges. In the fly *Drosophila*, Bridges found that an abnormality sometimes occurred when maturation of the egg was taking place; the two sex chromosomes in the immature egg did not part, so that as a consequence, either both passed out of the egg, or both remained within. The result was two types of abnormal eggs ready for fertilisation, one with two X chromosomes and one without any at all. Normal eggs have one.

Now, the male produces two kinds of spermatozoa, one with an X chromosome, and the other with a Y chromosome. If the first named fertilises a normal egg the result is a female with two X chromosomes, if the latter kind fertilises an egg a male results with an X and a Y chromosome. If, however, the sperm with a Y chromosome fertilises one of the abnormal eggs with two X chromosomes the result is a female, because it possesses two X chromosomes, whilst if the sperm with the X chromosome fertilises the abnormal egg with no sex chromosome at all, a male results.

The observations fit in beautifully with theoretical conceptions, and the enormous consequence of the sex chromosomes becomes evident.

How then do the conditions in the Honey Bee and many other Hymenoptera fit in with this theory of sex? It is well known, thanks in the first case to a German pastor and beekeeper, Pastor Dzierzon (1811-1906), that Queen Bees (the fully developed females) and Workers (females with undeveloped reproductive organs and other modifications) result from fertilised eggs, whereas Drones or males arise from eggs which develop without fertilisation at all—i.e., are parthenogenetic. Much dispute has arisen regarding this theory, but its accuracy as the normal process of sex determination has been established by the interesting experiments of Newell. It is difficult to observe the mating of the Honey Bee, because the act takes place during the nuptual flight.

Newell overcame this obstacle by taking his bees to Texas to a place where there were no other bees to invalidate his experiments. At this spot he brought together the Yellow Italian Bee and the Grey ~~Cariolarian~~ variety. When the yellow queens Carnio were crossed with the grey males, all the resulting progeny (males, workers and queens) were yellow. This, of course, indicates that in a cross between these two varieties, yellow is dominant to grey. The experiment throws no light on fertilisation, but is a necessary preliminary. When the opposite cross was made, however, and the grey queen was crossed with the yellow drone, the resulting generation consisted of grey males and yellow workers and queens. The grey males must have arisen from *unfertilised eggs* laid by the grey queen, otherwise they would have been yellow.

An examination of the cells of the male bee reveals the fact that the number of chromosomes present (16) is only half that of the queen (32). This big difference is only what might be expected from the fact that fertilisation has not taken place. It is not, however, this big difference which is important, but the fact that one can assume there is only one X chromosome present instead of two. In the mature egg of the queen bee there are 16 chromosomes, i.e., 15 + 1 X chromosome, and all the eggs are similar in this respect. In the spermatozoa of the male there are also 16 (the same number as in the other cells of the body, because there is no reduction division). Thus the fertilised eggs will contain 32 chromosomes, two of which are X chromosomes, whilst the unfertilised eggs will contain 16 chromosomes, only one being an X chromosome. It is the presence of the two X or only one X chromosomes which results in female or male development.

Thus, once again, the chromosome conditions fit in with the chromosome theory of sex. The special point of interest is that the bees do not employ the usual method (in which half the eggs or half the sperms are different from the others in regard to the chromosome garniture), but attain the same result by means of parthenogenesis. The normal mechanism of sex determination in the animal kingdom leaves the sex to chance, whilst in the Honey Bee one might almost say that the Queen had the ability to control it.*

Let us now regard the action of the chromosomes a little more closely. Recent discoveries show that the factors for the actual unfolding of both the male and female characters are inherited together. The egg has, in fact, the potentiality of developing both sets of characters. The function of the sex chromosomes seems then to be one of control. In other words, the sex chromosome mechanism decides which set of factors will prevail. And since both sets are present it is not surprising that sometimes both prevail, one following and repressing the other. A familiar example of this is the cock feathering of old hens, and Crew has shown recently how, in some cases, hens which were quite normal egg producers have developed male characters and even been capable of functioning as males. And so it is possible, notwithstanding the chromosome constitution, for variations to take place in the development of sexual characters. But in, perhaps, the majority of cases the ultimate sex depends upon the initial *quantitative* conditions of the sex chromosomes. This has been most beautifully demonstrated by Goldschmidt in experiments with different races of the Gipsy Moth. When normal breeding takes place the presence of only one X chromosome results in a female. It was found, however, that by crossing the male of what is termed a *weak* European race with the female of a *strong* Japanese race, a disharmony occurs ; the chromosome garniture that normally gives females, produces animals that are more or less mixed (sometimes almost completely male). These are termed intersexual females. They can be explained if we assume that the single sex chromosome which has come from the European male parent is not sufficiently potent to balance the female factors from the Japanese parent.

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* A description of the reproductive organs of the queen bee and the mechanism of fertilisation was given here by the lecturer.

The position stands then as follows : In the majority of cases a certain chromosome constitution determines the sex which will unfold itself during development. It is possible to disturb this by bringing chromosomes together which are not balanced in power, as for example, by crossing two races which have arisen far apart geographically and which normally do not breed together.

It is also possible to disturb the action of the chromosome mechanism by physico-chemical means which affect the functioning of the " machine." A partial realisation of the latter is also provided by the Honey Bee and as a matter of fact statements have been published to the effect that a complete realisation has been observed.

It is well known that worker bees are females (resembling the Queen in regard to chromosome garniture, but quite different in form and structure). Now, the workers can produce at will either a worker or a queen from a fertilised egg. So far as we know the influence exerted can only be by means of the food given to the bee larvae. Whatever this food contains, the form-determining influence is obviously chemical. And if it is possible to produce such differences as those between queen and worker it is not inconceivable that external influences might neutralise the tendency of the chromosomes and lead to intersexes or even to a complete sex change

During many years there has been some controversy in regard to sex in the bee which might be explained in the light of modern knowledge along these lines. For example, many beekeepers have asserted that at times males have arisen from fertilised eggs. (The reader is referred to the discussions and papers of Dickel, Breslau, Nachtstein, Cuénot and others.) It is true that much of the evidence is very unscientific—but there is usually fire where there is smoke and the whole question must be reopened as the result of a paper by Jack, describing certain experiments of G. W. Onions on the Cape Honey Bee. In this case worker bees produced females parthenogenetically.

It is, of course, possible that in these examples there have really been disturbances in the distribution of the chromosomes. But there are other examples which, indeed, show that the influence of the sex chromosomes on the development of sex characters can be modified. (Parasitic Castration, by *Sacculina.*)

We might sum up the present position as follows :—

(1) Every egg possesses the inherited potentiality of developing both the male and female characters.

(2) Which set of sex characters develops depends upon the metabolic conditions initiated by a certain chromosome garniture—the result of maturation and fertilisation or of maturation and parthogenesis (*Drosophila*, *Ancyracanthus*, the Honey Bee and many other cases).

(3) It is possible for this controlling mechanism to be disturbed by :—

(*a*) Upsetting the harmony of the chromosomes by crossing different races in which similar chromosomes are of different potency (Gipsy Moth crosses).

(*b*) The influence of external conditions which encourage or only permit of one type of metabolism. ("Parasitic Castration," by *Sacculina*.)

(*c*) The initial chemical constitution of the egg being such that a type of metabolism is favoured which is contrary to that favoured by the chromosomes. (Possibly Riddle's Pigeon Egg examples.)

NOTE.—It should be noted that the action of one set of sex factors may extend beyond the duration of the other or commence at a later period in the life of the animal. The disturbing influences stated above may decide the order of the reactions or may retard or accelerate differentiation of the embryo so that it occurs under the influence of one or of both sets of sex factors. (*Bonellia* and *Crepidula* and the Freemartin.)

(4) The chromosome garniture may itself in certain cases be the result of the action of the environment on maturing eggs (Seiler's experiments with Psychid Moths), or the result of an early occurring peculiarity in cell constitution. This does not invalidate the action of the sex chromosome mechanism. It implies, however, that the movements of the chromosomes are not altogether a matter of chance.

THE

MARINE BIOLOGICAL STATION AT PORT ERIN.

BEING THE

THIRTY-SIXTH ANNUAL REPORT

DRAWN UP BY

PROFESSOR JAMES JOHNSTONE, D.Sc.
(Department of Oceanography, University of Liverpool).

INTRODUCTION

During the past year the work of the Station has been satisfactory from every point of view. An account of various structural changes made during 1922 will be found in Mr. Bruce's Report on p. 14, and it will be seen that all possible efforts are being made to increase the general usefulness of the Laboratories. Difficulties and expenses resulting from the neglect imposed on us by the conditions of the war are gradually being overcome. An unexpected misfortune was the fall of a part of the cliff at the back of the Station, and this rather impeded some of the experimental work planned, as well as the conduct of the plaice hatching operations. The Insular Government most kindly took the expense of repairing the damage to the salt-water ponds, but other defects were discovered and these have been the occasion of much expense.

The financial condition is as satisfactory as can reasonably be expected. A statement will be found at pp. 36-37 of this Report. The ordinary income, supplemented most generously by the Development Commissioners and the Insular Government, has enabled us to carry on the work in a satisfactory manner. A somewhat large capital expenditure on a small vessel, with its equipment, and on some additional laboratory accommodation is still the chief desideratum from the point of view of continued expansion of the work of the station. Otherwise the present situation is satisfactory.

The educational work dominates the activity of the Institution to the extent that it has occasionally been a source of embarrassment in our efforts to expand the research side of the Station. The policy is, however, now an established one, and it would be most difficult, even if it were desirable, to make any change. The extent to which the Institution provides for University students of Zoology and Botany is evident from a glance at the Report drawn up by Mr. Bruce (pp. 14-22). It is evident that this work must be continued and even that further facilities may have to be given. Also the extent of the financial support given by the Universities will be assessed by a glance at the statement on p. 37. This support is not satisfactory, but it is clear that it cannot be increased in the meantime, and no further burden can be put upon the individual students or researchers. The highest types of educational work are always expensive and must be subsidised or original research will be hampered. One of our most important functions is to train young researchers.

RESEARCH WORK.

Several papers dealing with work done at the Station, have been published since the date of writing the last Annual Report. These are :—

B. Moore, F.R.S. (the late); E. Whitley, M.A.; and T. A. Webster.
"Studies of Photo-Synthesis in Marine Algæ."
(In the present Report.)

A. Scott, A.L.S.
"The Plankton of the Spawning-pond at Port Erin."
(In the present Report.)

E. C. Herdman (Miss).
"Notes on Dinoflagellates and other Organisms causing discolouration of the Sand at Port Erin." I and II.
(*Trans. Liverpool Biol. Soc.* Vol. 35, 1921; Vol. 36, 1922).

Ruth C. Bamber, M.Sc.

"Notes on Some Experiments on the Water Vascular system of Echinus."

(*Trans. Liverpool Biol. Soc.* Vol. 35, 1921.)

A. Scott, A.L.S.

"On the Food of Young Plaice."

(*Journ. Mar. Biol. Assn.* Vol. 12, No. 4, 1922.)

An "L.M.B.C. Memoir" on Asterias is now in the press and will be published shortly.

A detailed account of the winter and spring cod fishery in the Isle of Man in 1921-22 has been written by Mr. W. C. Smith, of the Oceanography Department at the University of Liverpool. This has been transmitted to the Ministry of Agriculture and Fisheries. It is part of the work done under a scheme of directed fishery research in the Irish Sea.

An investigation on the preservation of fish by means of chlorinated ice was made at the Station during June by Dr. W. Gibbs, of the Salt Union Research Department. Large scale tests on board steam trawlers were also made and the results have been very satisfactory. The product, ice impregnated with sodium hypochlorite, is now on the market under the name of "Salunol." Some interesting problems of scientific importance presented themselves in the course of this investigation, and further work is being done by Dr. Gibbs.

A series of specimens of cod collected at the station and illustrating the fishery of 1921-22 have been examined at the Department of Oceanography by Dr. Johnstone and Mr. R. A. Fleming. The quantities of water, proteid, fat and ash in each sample have been estimated at intervals throughout a yearly season and other observations have also been made. The results wait publication. (This is work done under the scheme of directed fishery research of the Ministry of Agriculture and Fisheries.)

A research on the oxygen consumption of developing plaice eggs was made at the station, during the spring spawning season of 1922, by Professor W. J. Dakin and his colleagues. This work is in progress.

Miss M. Knight has continued her work on the life-history of the Marine Alga, *Pylaiella*. The investigation is now nearly complete.

References to some other researches will be found in Appendix C (3) to this Report.

PROFESSOR BENJAMIN MOORE.

Some remarks may be made here on the paper on "Photosynthesis in Marine Algæ," which is published as an appendix to the present Report.

Its senior author, Professor Benjamin Moore, F.R.S., was a most valued supporter of the Port Erin Station, and his death has been the occasion of irreparable loss to our organisation. Moore came to Liverpool in 1902 as head of the newly-established Department of Bio-Chemistry—the first of its kind in this country. He became a member of the Liverpool Marine Biology Committee, and for many years he spent a considerable part of his University vacation working at the Station. There he developed a line of research that has proved to be stimulating and suggestive in the highest degree and which has led, through the work of Baly and Heilbron to a brilliant demonstration of the physico-chemical mechanism of photo-synthesis in the green plant, and has opened out prospects of illimitable practical significance. He greatly amplified the conception of the "Buffer Effect," which is due to certain salts and electrolytes in the blood plasma, extending this, in the most daring way, to study of the seasonal changes that occur in the sea during the period of organic production in the spring months. He studied the hypothesis of nutrition elaborated by Professor Putter, criticising it and suggesting further lines of investigation which we may expect to be taken up by marine bio-chemists in the near future. Some of this work was published in the Reports of the Lancashire Sea-Fisheries Laboratory and other papers were published by the Royal Society. In 1919, Moore and his co-workers, Edward Whitley and Arthur Webster, proceeded to a new subject—that of the assimilation of elementary nitrogen by marine algæ under the energising influence of sunlight, and here, again, development of this line of investigation is sure to be taken up with results of interest to science and practical importance in the economic sense. Both at the University and at Port Erin, Moore's influence on his colleagues was of the most valuable kind. He was essentially a man of ideas. In his own words he was " at the growing point " of every new movement, both in physical and in social science.

In 1914, he left Liverpool to join the newly-established Department of Applied Physiology set up by the Medical Research Council. The change was an unhappy one for pure science, and it was personally unfortunate. Moore was not the kind of man to take kindly to official traditions and methods. Still, the transfer was fortunate in another respect, for the work that he did on tri-nitro-toluene poisoning during the war years was extremely valuable and had results that have been fully recognised. In 1918, Moore left the Medical Research organization to become the first Professor in the Department of Bio-Chemistry at the University of Oxford, endowed by his friend, Edward Whitley. In 1919, he resumed his investigations at Port Erin, but even then he was in failing health. Ever since the death of his wife, a very talented lady, in 1911, he had been far from strong, and early in 1922 he fell ill with influenza. He died on March 10th of this year, at the age of 55 years.

The paper included in the Appendix contains the results of observations made at Port Erin in the spring of 1919 by Moore, Whitley, and Webster. During that period the very important subject of nitrogen assimilation suggested itself, and some work was done which was published in the Proceedings of the Royal Society in 1920. This led to the photo-synthetic results being set aside in the meantime. In 1920, the subject was taken up again, but criticism of the nitrogen-assimilation investigation had suggested new lines of enquiry, notably on the activities of certain species of bacteria which are said to be present in sea-water, on the sea bottom, and on the mucus that covers the surface of marine algæ. This, again, led to the postponement of the work on carbon-assimilation by algæ. It was intended that the observations here recorded should be extended and developed in some other directions, and plans for the future work were made. These cannot now be realised and so the results, imperfect as they are, are published in the hope that other hands will take up, and complete them.

Report of the Naturalist-in-Charge.

Seventy-four workers, making eighty-two separate visits, have this year occupied the laboratories.

Of these, the greater number have been undergraduate students from the Zoological and Botanical Departments of the Universities of Liverpool, Cambridge, Manchester, Wales (Aberystwyth and Bangor), and Bristol, and the University Colleges of Nottingham and Southampton. Besides these, however, a considerable number of advanced workers from the above and other institutions have carried out researches in the Biological and Bio-Chemical laboratories.

Professor W. J. Dakin made some interesting observations on the metabolism of newly-fertilised plaice eggs, and also, with Miss M. Hobbins, continued his work upon certain protozoan parasites of the common whelk. Miss M. S. C. Fordham devoted some attention to the Pycnogonida, and Miss O. R. Bangham, as Edward Forbes Exhibitioner, investigated the physiology of the water-circulatory system of *Echinus*.

Dr. W. E. Gibbs, Chief Chemist to the Salt Union, Ltd., devoted several weeks in the summer, to a study of the chemical changes occurring in fish stored in ordinary and in " treated " ice (" Salunol ").

Miss Knight has continued and practically completed her exhaustive study of the algal genus *Pylaiella*, and Miss E. C. Herdman has carried out work, embodied in a second paper, upon the occurrence and distribution of certain Dinoflagellates on the sandy shore of Port Erin Bay.

Professor Sir William Herdman has continued his observations upon the Plankton and sea-bottom fauna, while Mr. W. C. Smith and Mr. W. Birtwistle have made the Station a centre in their investigation of the Cod and Plaice Fisheries of the Irish Sea.

It is gratifying to note that the number of workers remains high, and that this year, the research activities have been spread over a longer period than usual.

The Fish Hatchery.

The most noteworthy and regrettable occurrence of the hatching season of 1922, was the demolition of the western spawning pond, on 1st February, by a great fall of rock from the cliff which rises abruptly from the southern parapet of the pond.

The Isle of Man Harbour Commissioners, as owners of the site, have undertaken the removal of the fallen stone, amounting to many hundreds of tons, and by further quarrying upon the cliff face, have minimised the risk of a recurrence of the disaster. They have also carried out repairs to the pond itself.

While this work has been in progress throughout the spring and summer, opportunity has been taken to carry out much needed improvements to the plan of the ponds, including the complete separation of the two portions of the East pond, and to repair the storage tanks. It is, however, probable that these improvements will not be appreciably reflected in the spawning output of the 1923 season, since the newness of the concrete is certain to prove deleterious to the fish.

The effect of the rock fall and consequent damage was to render useless at least one half of the spawning capacity of the plant, besides which, a number of spawning fish perished, and the energies of the staff were diverted, all at a critical period of the hatching season. Such measure of success as has attended upon our restricted operations is wholly due to the efforts of Mr. T. N. Cregeen, and his assistant, Mr. Wm. Christian, upon the former of whom the entire charge of the hatchery has devolved.

The stock of spawning plaice consisted almost entirely of new fish, caught locally, in Niarbyl, Fleshwick and Port Erin Bays, during the late autumn of 1921. The majority of the fish were carefully selected and well-developed specimens, averaging 2 lbs. in weight. No attempt was made to determine the proportion of the sexes.

It is believed that one hundred and eleven fish were alive at the opening of the spawning season in March.

Spawning extended throughout the whole of March and April, and the larvae were reared in a healthy condition.

The number of eggs collected, and of larvae set free, during the successive weeks of the hatching season is given below.

Week ending		Mean Temp. of pond, °F.	Eggs collected	Fertilised eggs (and larvae) set free
February	18th ...	40°·4	12,600	...
,,	25th ...	42°·0	118,650	...
March	4th ...	43°·8	127,050	...
,,	11th ...	42°·9	175,350	31,500
,,	18th ...	42°·3	646,800	79,800
,,	25th ...	39°·6	1,071,000	63,000
April	1st ...	39°·7	1,228,500	247,800
,,	8th ...	40°·0	1,971,900	466,200

Week ending		Mean Temp. of pond, °F.	Eggs collected	Fertilised eggs (and larvae) set free
April	15th ...	42°·1	970,200	224,700
,,	22nd ...	45°·2	321,300	2,143,050
,,	29th ...	46°·4	107,100	1,956,150
May	6th ...	48°·5	29,400	579,600
,,	13th ...	53°·0	...	174,300
,,	20th ...	54°·5	...	22,050
			6,779,850	5,988,150

It may be of interest to note that, since the commencement of hatching operations in 1904, upwards of 94 millions of larval plaice have been set free in the coastal waters of the Isle of Man.

Lobster Culture.

The limitations imposed by the rock fall, referred to under the hatchery reports, weighed even more seriously upon the success of lobster culture.

As it was not possible to use the outdoor pond, the "berried" female lobsters were placed in the uppermost boxes of the hatching tanks—a method previously attempted and found not to be very successful.

Thirteen female lobsters only were obtained, and these, during the months of July and August, yielded 5,800 larvae. Of these, about 1,000 were placed in the rearing jars, but rather less than 13 per cent. were reared to the "lobsterling" or fourth stage. The remaining 4,800 larvae were liberated in the first and second stages.

The Aquarium.

The Aquarium has continued to attract large numbers of summer visitors; 28,859 persons have paid for admission during the year, and this number includes nearly 3,500 children. In addition to the above, several parties of scholars from the Insular schools have been admitted free of charge, and are not included in the above figure.

The display of local fishes in the wall-tanks has been very well maintained. The majority of these have been caught by line or trammel-net, by the station staff, and in nearly every case have survived captivity in a healthy condition.

A list of the principal fishes placed on exhibition during the year follows, and those which are alive at the time of this report (November) are distinguished by an asterisk :—

*Angler-fish (_Lophius piscatorius_)
*Brill (_Rhombus laevis_)
*Cod (_Gadus callarias_)
*Coal-fish (_Gadus virens_)
Conger (_Conger vulgaris_)
*Dab (_Pleuronectes limanda_)
*Dogfish, Spotted (_Scyllium canicula_)
*Gurnard, Grey (_Trigla gurnardus_)
*Gurnard, Sapphirine (_Trigla cuculus_)
John Dory (_Zeus faber_)
*Lemon Sole (_Pleuronectes microcephala_)
*Mackerel (_Scomber scomber_)
Müller's Top-knot (_Zeugopterus punctatus_)
*Plaice (_Pleuronectes platessa_)
*Pollack (_Gadus pollachius_)
*Ray, Thornback (_Raia clavata_)
*Sole (_Solea vulgaris_)
*Sea-bream (_Pagellus centrodontus_)
*Sea-scorpion (_Cottus scorpius_)
*Whiting-pout (_Gadus luscus_)
*Wrasse (_Labrus mixtus_)
*Whiting (_Gadus merlangas_)

A number of rock-pool fishes were also shown, in smaller tanks, as well as the usual invertebrates in the shallow table-tanks.

A selection of publications of the Station and the Department were placed on view, and a considerable number of "L.M.B.C. Memoirs," Annual Reports, Maps, etc., have been sold to the public, as well as 835 copies of the "Guide to the Aquarium," thereby practically exhausting the fourth edition.

The Library.

The Library has shared, with the rest of the building, in the general scheme of reconditioning.

The shelving has been altered, so as to facilitate access to the books, and the room has been suitably decorated.

Miss Allen spent some days, at Easter, in completing and extending the card-index begun by Mr. Chadwick. The large collection of pamphlets and separata has been classified, and placed in a series of filing-cases.

As in past years, a number of Institutions and Fisheries Boards have presented copies of their publications, and several works, including a number upon physiological and bio-chemical subjects, have been purchased.

The Bio-Chemical Laboratory.

The temporary laboratory and store-room adjacent to it, immediately above the Fish Hatchery, have been thrown into one, and the large, airy, and well-lighted apartment thus formed, 35 feet by 25 feet, has been fitted out with benches, shelving, sinks, and gas, as a chemical laboratory. This laboratory is designed, primarily, for research purposes, and is capable of accommodating six or eight workers.

A supply of general apparatus and reagents, suitable for ordinary physiological and bio-chemical operations, is available, but special pieces of apparatus will be obtained, from time to time, as the need for them arises, and as researchers present themselves.

It is hoped that the facilities thus provided for seaside research in the special province of the bio-chemist will be widely made use of, and that a new school of bio-chemists will take up and extend the work which, under far less advantageous conditions, was begun by Professor Benjamin Moore and his co-workers, and interrupted by his lamented death.

Intending workers in the bio-chemical laboratory should apply to the Director, Oceanography Department, University of Liverpool, for particulars.

Other Work carried out during 1922, *to the Station premises, etc.*

Laying of pipe line, direct from the Storage Tanks to the Tank Room, with facilities for connecting up, at a later date, with the Aquarium supply pipe. Included in this scheme is the provision of a permanent water-supply to the experimental tanks at the foot of the lower storage tank, and to the Photographic Tank, and of a shallow cemented sink and drainage-basin, by the Hatchery door.

A complete overhaul and reconditioning of the oil engine, petrol engine, and pumps.

Series of public scientific lectures, arranged in conjunction with the George Herdman Institute Committee, during the winter season, 1922-23.

List of Workers, 1922.

March 20	to	April 1	Miss E. M. Roper, Liverpool U. Marine Algæ.
,,	,,	,,	Miss E. M. Thomas, Liverpool U. Marine Algæ.
,,	,,	,,	Miss May Allen, Liverpool U. Library.
,,	,,	March 25	Mr. H. Hamshaw Thomas, Botany School, Cambridge. Marine Algæ.
,,	,,	April 5	Mr. S. M. Wadham, Botany School, Cambridge. Marine Algæ.
,,	,,	,,	Mr. M. A. H. Tincker, Botany School, Cambridge. Marine Algæ.
,,	,,	,,	Mr. R. D. Good, Botany School, Cambridge. Marine Algæ.
,,	,,	,,	Mr. C. Rimington, Botany School, Cambridge. Marine Algæ.
,,	,,	,,	Miss E. G. Torrance, Botany School, Cambridge. Marine Algæ.
,,	,,	,,	Miss E. M. Young, Botany School, Cambridge. Marine Algæ.
March 22	,,	March 25	Prof. Jas. Johnstone (Director), Liverpool U. Official
,,	,,	April 24	Prof. Sir Wm. A. Herdman, Liverpool U. Plankton and General.
,,	,,	,,	Miss E. C. Herdman, Liverpool U. Ascidians, Flagellates.
March 24	,,	April 3	Mr. P. E. Pieris, Cambridge. Gen. Marine Biol.
,,	,,	April 22	Prof. W. J. Dakin, Liverpool U. Exptl. Physiol.
,,	,,	,,	Miss M. S. C. Fordham, Liverpool U. Pycnogonida.
March 25	,,	,,	Miss W. Kehoe, Liverpool U. Exptl. Physiol.
,,	,,	,,	Miss E. Angel, Liverpool U. Exptl. Physiol.
March 27	,,	April 5	Mr. C. W. D. Kermode, Botany School, Cambridge. Marine Algæ.
March 30	,,	April 11	Miss M. E. Holder, Manchester U. Marine Zoology.
,,	,,	,,	Miss Rankine, Manchester U. Marine Zoology.

March 30	to	April 20	Dr. W. Ritchie, Univ. Coll., Aberystwyth, Marine Zoology.
,,	,,	April 13	Mr. D. O. Morgan, Univ. Coll., Aberystwyth. Marine Zoology.
,,	,,	,,	Mr. E. E. Watkins, Univ. Coll., Aberystwyth. Marine Zoology.
,,	,,	,,	Mr. B. D. Burtt, Univ. Coll., Aberystwyth. Marine Zoology.
March 31	,,	April 22	Miss I. A. F. Hilton, Liverpool U. Marine Zoology.
,,	,,	,,	Miss E. Lowe, Liverpool U. Marine Zoology.
April 1	,,	April 4	Mrs. Bisbee, Liverpool U. Marine Zoology.
April 3	,,	April 11	Miss D. Bexon, Univ. Coll., Nottingham. Gen. Marine Biol.
,,	,,	,,	Mr. T. Harris, Univ. Coll., Nottingham. Gen. Marine Biol.
,,	,,	,,	Mr. R. Burgess, Univ. Coll., Nottingham. Gen. Marine Biol.
,,	,,	,,	Mr. Gee, Univ. Coll., Nottingham. Gen. Marine Biol.
,,	,,	April 21	Miss M. Hobbins, Liverpool U. Protozoa, Parasites.
April 4	,,	April 20	Miss E. M. Staidler, Liverpool U. Marine Zoology.
,,	,,	April 22	Miss Wilson, Liverpool U. Marine Algæ.
April 6	,,	,,	Miss J. McInnes, Liverpool U. Marine Algæ.
,,	,,	,,	Miss M. Ryder, Liverpool U. Marine Algæ.
,,	,,	,,	Miss K. M. Huyton, Liverpool U. Marine Algæ.
,,	,,	,,	Miss D. A. S. Hughes, Liverpool U. Marine Zoology.
,,	,,	April 25	Miss O. R. Bangham, Liverpool U. Physiol. of Echinus.
April 8	,,	April 22	Miss E. L. Gleave, Oulton School, Liverpool. Gen. Marine Biol.
,,	,,	,,	Miss E. Christian, Oulton School, Liverpool. Gen. Marine Biol.
,,	,,	,,	Miss M. Simpson, Liverpool U. Exptl. Physiol.
,,	,,	,,	Miss F. Hey, Liverpool U. Exptl. Physiol.
,,	,,	April 18	Miss L. Thorpe, Univ. Coll., Bangor. Gen. Marine Biol.
,,	,,	,,	Miss L. M. Thomas, Univ. Coll., Bangor. Gen. Marine Biol.
,,	,,	,,	Mr. W. R. Wright, Univ. Coll., Bangor. Gen. Marine Biol.
,,	,,	,,	Mr. R. Edwards, Univ. Coll., Bangor. Gen. Marine Biol.
April 10	,,	April 19	Miss W. R. Frost, Liverpool U. Marine Zoology.
,,	,,	,,	Miss M. L. Jones, Liverpool U. Marine Zoology.
,,	,,	,,	Miss E. J. Rutherford, Liverpool U. Marine Zoology.
,,	,,	April 22	Miss J. J. Anderton, Liverpool U. Marine Zoology.
,,	,,	,,	Miss G. R. L. Lishman, Liverpool U. Marine Zoology.
,,	,,	,,	Miss M. McCartan, Liverpool U. Marine Zoology.
,,	,,	,,	Miss E. M. Owen, Liverpool U. Marine Zoology.
,,	,,	,,	Miss K. E. Phillips, Liverpool U. Marine Zoology.
,,	,,	,,	Miss M. M. Ryan, Liverpool U. Marine Zoology.
,,	,,	,,	Miss D. M. Schulthess, Liverpool U. Marine Zoology.
April 13	,,	April 19	Miss Brown, Liverpool U. Marine Algæ.
,,	,,	,,	Miss Doran, Liverpool U. Marine Algæ.
April 19	,,	April 22	Dr. B. Prashad, Indian Zool. Survey. Mollusca and General.

June 3	to	July 24	Dr. W. E. Gibbs, Salt Union, Ltd. Fish Preservation.
June 7	,,	June 9	Prof. Sir Wm. A. Herdman, Liverpool U. General.
,,	,,	,,	Miss E. C. Herdman, Liverpool U. Ascidians, Flagellates.
June 12	,,	July 15	Miss G. Pickford, Newnham, Cambridge. Marine Zoology.
July 4	to	Sept. 4	Miss M. Knight, Liverpool U. Algæ—Pylaiella.
July 6	,,	Aug. 9	Prof. Sir Wm. A. Herdman, Liverpool U. Plankton, General.
,,	,,	,,	Miss E. C. Herdman, Liverpool U. Ascidians, Flagellates.
,,	,,	,,	Miss D. Eyden, Newnham, Cambridge. Marine Zoology.
July 14	,,	Sept. 4	Miss Hanson, Liverpool U. General.
July 17	,,	July 28	Miss G. Eyres, Bristol U. Marine Zoology.
,,	,,	July 22	Mr. W. C. Smith, Liverpool U. Cod Fisheries.
July 19	,,	Aug. 4	Dr. W. G. N. van der Sleen, Haarlem. Marine Zoology.
,,	,,	,,	Mr. W. L. Varossieau, The Hague. Marine Zoology.
,,	,,	,,	Miss T. S. S. van Benthem Jutting, Amsterdam. Marine Zoology.
,,	,,	July 20	Prof. Jas. Johnstone (Director), Liverpool U. Official.
Aug. 10	,,	Aug. 17	Miss M. Spencer, Univ. Coll., Southampton, Marine Algæ.
Sept. 16	,,	Sept. 30	Prof. Sir Wm. A. Herdman, Liverpool U. Plankton, General.
,,	,,	,,	Miss E. C. Herdman, Liverpool U. Ascidians, Flagellates.
Sept. 19	,,	Sept. 26	Mr. W. Birtwistle, Liverpool U. Plaice, Icthyometry.
,,	,,	Oct. 3	Mrs. Bisbee, Liverpool U. Marine Zoology.
Sept. 20	,,	Sept. 26	Mr. W. C. Smith, Liverpool U. Cod, Icthyometry.

(TOTAL—82 visits by 74 workers).

J. RONALD BRUCE.

THE EDWARD FORBES EXHIBITION.*

The Exhibitioner for the year 1922 was Miss O. R. Bangham She worked, in collaboration with Professor Dakin, on the physiology of the water vascular system of *Echinus*. The results of the investigation are still incomplete.

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* See pp. 30-31 for the Regulations with regard to this Exhibition.

FAUNISTIC NOTES.

Laophonte proxima, G. O. Sars. One or two specimens of this copepod were found while examining the plankton of the pond. It has not previously been recorded outside the Norwegian coast and is an addition to the extensive list drawn up by the old L.M.B.C.

Cerianthus lloydii, Gosse. The discovery of an extensive colony of this apparently rare tube-building anemone was described in last year's Annual Report. It has now been found to occur on both sides of the entrance to Barrow Channel in considerable numbers. I suggested in the report that it would be interesting to know if the colony discovered by Mr. Alfred Lloyd in the Menai Straits in 1865 still existed. It evidently does still persist. Shortly after the publication of the report, Mr. Chadwick received a post card from one of his correspondents in the University of Manchester and sent it on to me. I think it is worth placing on record :—

"*March* 31, 1922.

Dear Mr. Chadwick,

I have been reading Mr. Scott's article on *Cerianthus lloydii*, Gosse, in your publication. You might let him know that I saw *Cerianthus lloydii* in the Straits a few years ago when I was staying at Penmon in Anglesea. It was not at all uncommon.

With kind regards,
Yours faithfully,
(*signed*) SCHAFFERS."

Polycera quadrilineata. This nudibranch, which was so abundant on the *Halidrys* bed on Piel Island shore last year, was only represented by a single specimen in 1922. Of the other species mentioned in last year's report only *Archidoris* and *Dendronotus* could be found.

A. SCOTT.

"L.M.B.C. MEMOIRS."

Although the Liverpool Marine Biology Committee has now been dissolved, it is thought well to retain the former title for this series of publications. They have become well known in laboratories and are referred to in literature as the "L.M.B.C. Memoirs," and it would only lead to confusion to change the title, although they are no longer published by a Committee but by the Oceanography department of the University.

Since the last report was published, the Memoir on APLYSIA has been issued to the public. ASTERIAS, by H. C. Chadwick, is in the printer's hands and will be published shortly; Miss E. L. Gleave, M.Sc., has nearly completed her Memoir on DORIS, the Sea-lemon; Mr. Burfield, is writing the Memoir on SAGITTA; Mrs. Bisbee has made further progress with TUBULARIA; Miss E. C. Herdman is far advanced with BOTRYLLUS, and still other Memoirs are in preparation.

The following shows a list of the Memoirs already published or arranged for:

I. ASCIDIA, W. A. Herdman, 60 pp., 5 Pls.
II. CARDIUM, J. Johnstone, 92 pp., 7 Pls.
III. ECHINUS, H. C. Chadwick, 36 pp., 5 Pls.
IV. CODIUM, R. J. H. Gibson and H. Auld, 3 Pls.
V. ALCYONIUM, S. J. Hickson, 30 pp., 3 Pls.
VI. LEPEOPHTHEIRUS AND LERNÆA, A. Scott, 5 Pls.
VII. LINEUS, R. C. Punnett, 40 pp., 4 Pls.
VIII. PLAICE, F. J. Cole and J. Johnstone, 11 Pls.
IX. CHONDRUS, O. V. Darbishire, 50 pp., 7 Pls.
X. PATELLA, J. R. A. Davis and H. J. Fleure, 4 Pls.
XI. ARENICOLA, J. H. Ashworth, 126 pp., 8 Pls.
XII. GAMMARUS, M. Cussans, 55 pp., 4 Pls.
XIII. ANURIDA, A. D. Imms, 107 pp., 8 Pls.
XIV. LIGIA, C. G. Hewitt, 45 pp., 4 Pls.
XV. ANTEDON, H. C. Chadwick, 55 pp., 7 Pls.
XVI. CANCER, J. Pearson, 217 pp., 13 Pls.
XVII. PECTEN, W. J. Dakin, 144 pp., 9 Pls.
XVIII. ELEDONE, A. Isgrove, 113 pp., 10 Pls.
XIX. POLYCHAET LARVÆ, F. H. Gravely, 87 pp., 4 Pls.
XX. BUCCINUM, W. J. Dakin, 123 pp., 8 Pls.

XXI. EUPAGURUS, H. G. Jackson, 88 pp., 6 Pls.
XXII. ECHINODERM LARVÆ, H. C. Chadwick, 40 pp., 9 Pls.
XXIII. TUBIFEX, G. C. Dixon, 100 pp., 7 Pls.
XXIV. APLYSIA, Nellie Eales, 84 pp., 7 Pls.
XXV. ASTERIAS, H. C. Chadwick.
DORIS, E. L. Gleave.
TUBULARIA, R. C. Bisbee.
SAGITTA, S. T. Burfield.
OSTRACOD (CYTHERE), A. Scott.
BOTRYLLUS, E. C. Herdman.
NEMATODE, T. Southwell.

As the result of a slight fire in the Zoology Department of the University, a portion of the stock of L.M.B.C. Memoirs has been partially destroyed. There are a certain number of damaged copies of some of the Memoirs which are stained or singed externally, but are still quite usable, and are suitable for laboratory work. It has been decided to offer these at prices ranging according to the condition from one-half to one-fourth of the published prices, as follows:—Memoir I., Ascidia, 6d. to 9d.; VI., Lepeophtheirus and Lernæa, 6d. to 1s.; VII., Lineus, 6d. to 1s.; XIII., Anurida, 1s. to 2s.; XIV., Ligia, 6d. to 1s.; XV., Antedon, 6d. to 1s. 3d.

Memoirs should be ordered from the University Press, Ashton Street, Liverpool.

Appended to this Report are:—

(A) The Laboratory Regulations—with Memoranda for the use of students, and the Regulations in regard to the "Edward Forbes Exhibition" at the University of Liverpool;

(B) The Financial Statement, List of Subscribers, and Balance Sheet for the year.

(C) Two papers:—

(1) On Photosynthesis in Marine Algae, by the late B. Moore, E. Whitley, and T. A. Webster.

(2) On the Plankton of the Spawning Pond, by A. Scott.

(3) List of other Published Works.

Appendix A

LIVERPOOL MARINE BIOLOGICAL STATION
AT
PORT ERIN.

General Regulations.

I.—This Biological Station is under the control of the Oceanography department of the University of Liverpool, and the Director of the Laboratory is the Professor of Oceanography.

II.—In the absence of the Director, the Station is under the temporary control of the Naturalist-in-Charge (Mr. J. R. Bruce, M.Sc.), who will keep the keys, and will decide, in the event of any difficulty, which places are to be occupied by workers, and how the tanks, boats, collecting apparatus, etc., are to be employed.

III.—The Naturalist-in-Charge will be ready at all reasonable hours and within reasonable limits to give assistance to workers at the Station, and to do his best to supply them with material for their investigations.

IV.—Visitors will be admitted, on payment of a small specified charge, at fixed hours, to see the Aquarium and Museum adjoining the Station. Occasional public lectures are given in the Institution by members of the staff.

V.—Those who are entitled to work in the Station, when there is room, and after formal application to the Director, are :—(1) Annual Subscribers of one guinea or upwards to the funds (each guinea subscribed entitling to the use of a work place for three weeks), and (2) others who are not annual subscribers, but who pay the University 10s. per week for the accommodation and privileges. Institutions, such as Universities and Museums, may become subscribers in order that a work place may be at the disposal of their students or staff for a certain period annually; a subscription of two guineas will secure a work place for six weeks in the year, a subscription of five guineas for four months, and a subscription of £10 for the whole year.

VI.—Each worker is entitled to a work place opposite a window in the Laboratory, and may make use of the microscopes and other apparatus, and of the boats, dredges, tow-nets, &c., so far as is compatible with the claims of other workers, and with the routine work of the Station.

VII.—Each worker will be allowed to use one pint of methylated spirit per week free. Any further amount required must be paid for. All dishes, jars, bottles, tubes, and other glass may be used freely, but must not be taken away from the Laboratory. Workers desirous of making, preserving, or taking away collections of marine animals and plants, can make special arrangements with the Director in regard to bottles and preservatives. Although workers in the Station are free to make their own collections at Port Erin, it must be clearly understood that (as in other Biological Stations) no specimens must be taken for such purposes from the Laboratory stock, nor from the Aquarium tanks, nor from the steam-boat dredging expeditions, as these specimens are the property of the Institution. The specimens in the Laboratory stock are preserved for sale, the animals in the tanks are for the instruction of visitors to the Aquarium, and as all the expenses of steamboat dredging expeditions are defrayed from the funds, the specimens obtained on these occasions must be retained (*a*) for the use of the specialists working at the Fauna of Liverpool Bay, (*b*) to replenish the tanks, and (*c*) to add to the stock of duplicate animals for sale from the Laboratory.

VIII.—Each worker at the Station is expected to prepare a short report upon his work—not necessarily for publication—to be forwarded to the Director before the end of the year for notice, if desirable, in the Annual Report.

IX.—All subscriptions, payments, and other communications relating to finance, should be sent to the Accountant, the University of Liverpool. Applications for permission to work at the Station, or for specimens, or any communications in regard to the scientific work should be made to the Director, Department of Oceanography, University, Liverpool.

MEMORANDA FOR STUDENTS AND OTHERS WORKING AT THE PORT ERIN BIOLOGICAL STATION.

Post-graduate students and others carrying on research will be accommodated in the small work-rooms of the ground floor laboratory and in those on the upper floor of the new research wing. Some of these little rooms have space for two persons who are working together, but researchers who require more space for apparatus or experiments will, so far as the accommodation allows, be given rooms to themselves.

Undergraduate students working as members of a class will occupy the large laboratory on the upper floor or the front museum gallery, and it is very desirable that these students should keep to regular hours of work. As a rule, it is not expected that they should devote the whole of each day to work in the laboratory, but should rather, when tides are suitable, spend a portion at least of either forenoon or afternoon on the sea-shore collecting and observing.

Occasional collecting expeditions are arranged under guidance either on the sea-shore or out at sea, and all undergraduate workers should make a point of taking part in these.

It is desirable that students should also occasionally take plankton gatherings in the bay for examination in the living state, and boats are provided for this purpose at the expense of the Biological Station to a reasonable extent. Students desiring to obtain a boat for such a purpose must apply to the Curator at the Laboratory for a boat voucher. Boats for pleasure trips are not supplied by the Biological Station, but must be provided by those who desire them at their own expense.

Students requiring any apparatus, glass-ware or chemicals from the store-room must apply to the Curator. Although a few microscopes are kept at the Biological Station, these are mainly required for the use of the staff or for general demonstration purposes. Students are therefore strongly advised, especially during University vacations, not to rely upon being able to obtain a suitable microscope, but ought if possible to bring their own instruments.

Students are advised to provide themselves upon arrival with the "Guide to the Aquarium" (price 6d.), and should each also buy a copy of the set of Local Maps (price 2d.) upon which to insert their faunistic records and other notes.

Occasional evening meetings in the Biological Station for lecture and demonstration purposes will be arranged from time to time. Apart from these, it is generally not advisable that students should come back to work in the laboratory in the evening; and in all cases all lights will be put out and doors locked at 10 p.m. When the institution is closed, the key can be obtained, by those who have a valid reason for entering the building, only on personal application to the Naturalist-in-Charge.

REGULATIONS OF THE EDWARD FORBES EXHIBITION.

[Extracted from the *Calendar* of the University of Liverpool for the Session 1920-21, p. 427.]

"EDWARD FORBES EXHIBITION.

"Founded in the year 1915 by Professor W. A. Herdman, D.Sc., F.R.S., to commemorate the late Edward Forbes, the eminent Manx Naturalist (1815-1854), Professor of Natural History in the University of Edinburgh, and a pioneer in Oceanographical research.

The Regulations are as follows :—

(1) The interest of the capital, £100, shall be applied to establish an Exhibition which shall be awarded annually.

(2) The Exhibitioner shall be a post-graduate student of the University of Liverpool, or, in default of such, a post-graduate student of another University, qualified and willing to carry on researches in the Manx seas at the Liverpool Marine Biological Station at Port Erin, in continuation of the Marine Biological work in which Edward Forbes was a pioneer.

(3) Candidates must apply in writing to the Registrar, on or before 1st February.

(4) Nomination to the Exhibition shall be made by the Faculty of Science on the recommendation of the Professor of Zoology.

(5) The plan of work proposed by the Exhibitioner shall be subject to the approval of the Professor of Zoology.

(6) Should no award be made in any year, the income shall be either added to the capital of the fund, or shall be applied in such a way as the Council, on the recommendation of the Faculty of Science, may determine.

(7) The Council shall have power to amend the foregoing Regulations, with the consent of the donor, during his lifetime, and afterwards absolutely; provided, however, that the name of Edward Forbes shall always be associated with the Exhibition, and that the capital and interest of the fund shall always be used to promote the study of Marine Biology."

Edward Forbes Exhibitioners.

1915	Ruth C. Bamber, M.Sc.
1916	E. L. Gleave, M.Sc.
1917	C. M. P. Stafford, B.Sc.
1918	Catherine Mayne, B.Sc.
1919	George Frederick Sleggs, B.Sc.
1920	Laura Thorpe, B.Sc.
1921	Maisie Hobbins, B.Sc.
1922	Miss O. R. Baugham, B.Sc.

Appendix B.

On August 1st, 1922, the assets of the Station were as follows :—

(1) The lease of the buildings and land of the Station. These were transferred to the University on 5th July, 1920, by an Act of the Manx Legislature. The internal equipment of the laboratories and hatchery. The Library : an inventory with estimates of the value of the equipment is in preparation.

(2) The investments, amounting in all to £989 14s. 3d.

(3) Cash in the bank and in hand, amounting to £499 6s. 1d.

The income from all sources, except grants from Government, during the year in question, was made up as follows :—

Interest, £53 15s. 1d. ; subscriptions, donations, and hire of work tables, £86 14s. 0d. ; admissions to the Aquarium, £368 19s. 4d. ; sale of specimens, publications, etc., £82 4s. 10d. ; a balance carried over from a building extension fund, £37 4s. 9d. ; miscellaneous sums, £12 15s. 0d. ; Total, £841 13s. 0d.

The Government grants for the year 1st August, 1921, to 31st July, 1922, were as follows :—

From the Development Fund, £921 8s. 4d. ; from the Isle of Man Treasury, £200 ; Total, £1,121 8s. 4d.

The expenditure on all objects is shown in the annexed statement ; the total was £1,861 16s. 1d. There is, therefore, an apparent deficit. The University financial year is, however, 1st August to 31st July following, while the Government financial year is 1st April to 31st March following. In respect of the ordinary accounting, the University year is followed, but in respect of the two official grants the Government year must be taken into account. The result is distracting, and it involves an unceasing waste of time. It is impossible to unravel the matter here. A glance at the balance sheet shows a balance in hand, at the end of the University year, of £499 6s. 1d. This, however, is only the approximate balance since, on the one hand, not all of the instalment of the Development Fund grant had been spent at that date, whereas, on the other, " requisitions " for salaries to the end of September had been made and counted as expenditure in the University system of book-keeping. At 1st August, the actual *estimated* balance would be about £400. This, of course, included the balance of the " Memoir Fund," which is available only for printing the " L.M.B.C. Memoirs."

Putting the whole matter in a simple, reasonable way, such as will convey information, we find :

The Station exists rent-free, though there is a small charge for rates and taxes.

It has an income, from its investments, of about £50 a year.

It can depend on a sum of about £450 a year from admissions to the Aquarium, sales of specimens, guides, and memoirs.

It obtains, at present, about £50 a year from voluntary subscriptions.

It has an income of about £40 a year from various Universities, being the hire of work-places in the laboratories.

There are other small fees, etc. Altogether, the assured " private " income is about £600 a year. From time to time there are donations, but these cannot be regarded as assured.

The official grants at present are £900 (approx.) from the Development Fund and £200 from the Isle of Man Treasury. These grants are, of course, renewable from year to year. They may be discontinued, and there can be no assurance that they will be paid in the future. Plans of regulated expenditure, calculated for the slow but reasoned and progressive development of the resources of the Station, cannot therefore be made. At the best, the arrangements must be " hand-to-mouth " ones, quite provisional, " unscientific," and thoroughly unsatisfactory.

Some additional information is given in last year's report on the finance of the Station. I append here—

(1) A list of the subscribers, and

(2) The University accounts as certified by the auditors.

THE UNIVERSITY OF LIVERPOOL

OCEANOGRAPHY: PORT ERIN ACCOUNT.

LIST OF SUBSCRIPTIONS TO 31st JULY, 1922.

	£	s.	d.
Brunner, Sir J. F. L., 43, Harrington-gardens, London, S.W.	2	2	0
Herdman, Professor Sir William, Croxteth Lodge, Ullet-road	2	2	0
Hickson, Professor A. M, The University of Manchester	2	2	0
Holt, Miss Sudley, Mossley Hill, Liverpool ...	2	2	0
Halls, W. J., 2, Townfield-road, West Kirby ...	2	2	0
Hutton, J. A., Woodlands, Alderley Edge ...	1	0	0
The Isle of Man Natural History Society, Castletown, I.O.M.	2	2	0
Jarmay, Sir John, Hartford, Cheshire	1	1	0
The Manchester Microscopical Society, 8 Daimler-street, Alms Hill-road, Manchester ...	1	1	
Mond, R., Sevenoaks, Kent...	5	0	0
Mond, R. C.	2	0	0
Muspratt, Dr. E. K., Seaforth Hall, Liverpool ...	5	0	0
Nuttall, F. R. Dixon, Ingleholme, Eccleston Park, Nr. Prescot	2	2	0
Rathbone, Miss M., 48, Norfolk-square, London, W.2	1	1	0
Roberts, Mrs. Isaac, Thomery, S. et M., France ...	1	1	0
Robinson, Miss M. E., Holmfield, Aigburth, L'pool	1	0	0
Smith, A. T., 43, Castle-street, Liverpool... ...	1	1	0
Thornely, Miss, Field Head, Out Gate, Ambleside	2	2	0
Toll, J. M., 49, Newsham-drive, Liverpool ...	1	1	0
University College of North Wales	2	0	0
Walker, A. O., Ulcombe-place, Maidstone ...	1	1	0
Watson, A. T., Tapton Crescent-road, Sheffield ...	1	1	0
Whitley, E., 13, Linton-road, Oxford	5	0	0
	£46	4	0

PORT ERIN BIOLOGICAL STATION.

BALANCE SHEET, 31 JULY, 1922

LIABILITIES

	£ s. d.	£ s. d.
99 Years Lease of Port Erin Biological Station and Fish Hatchery from 5 July, 1920 ... ... (*Vide The Biological Station and Fish Hatchery Transfer Act*, 1920)		per contra
Donations:—		
Transfer from Liverpool Marine Biological Committee	909 14 3	
Mr. John Rankin	80 0 0	
		989 14 3
General Account		310 0 1
Memoir Fund		131 1 2
Development Fund		58 4 10
		£1489 0 4

We have examined the above Balance Sheet and certify that it is correct.

HARMOOD BANNER & SON,

LIVERPOOL, 28 *August*, 1922. *Chartered Accountants.*

ASSETS

	£ s. d.	£ s. d.
99 Years Lease of Port Erin Biological Station and Fish Hatchery from 5 July, 1920 ... ... (*Vide The Biological Station and Fish Hatchery Transfer Act*, 1920)		per contra
Investments at Cost:—		
99 Shares in British Workman Public House Company, Limited	121 5 6	
Funding Loan 4 %, 1960-1990, £1135 16s. 7d.	788 8 9	
Victory Bond 4 %, £100	80 0 0	
		989 14 3
Cash in Isle of Man Banking Company, Limited, Port Erin Branch	449 6 1	
Cash in hands of Curator	50 0 0	
		499 6 1
		£1489 0 4

C. SYDNEY JONES,

Treasurer.

General Account

PAYMENTS		£ s. d.
To Balance—Deficit, 1 August, 1921		2 9 5
,, Salaries (including Insurance)—		
Assistant Curator	250 0 0	
Assistants	49 5 0	
		299 5 0
,, Fuel, Light, Cleaning, &c.		101 9 5
,, National Insurance (Employer's Contributions)		1 16 8
,, Boat hire		8 5 0
,, Rates, Taxes, Insurance		25 3 9
,, Carriage and Cartage		10 3 4
,, Contribution to Superannuation Fund		43 6 8
,, Balance—Credit to Balance Sheet		310 0 1
		£801 19 4

RECEIPTS		£ s. d.
By Interest on Investments		47 11 10
,, Subscriptions and Donations		46 4 0
,, Universities, Hire of Work Tables		40 10 0
,, Port Erin:—		
Admissions to Aquarium	368 19 4	
Sales of Guides and Postcards	26 4 10	
Sales of Specimens	16 6 4	
Laboratory Fees	11 7 0	
		422 17 6
,, Rebate on Petrol Duty		1 8 0
,, Grant from I.o.M. Treasury		200 0 0
,, Bank Interest		6 3 3
,, Balance of Extension Fund		37 4 9
		£801 19 4

Memoir Fund Account

To Expenditure on Printing, etc.	£67 0 0
,, Balance—Credit to Balance Sheet	131 1 2
	£198 1 2

By Balance—1 August, 1921	£158 7 6
,, Sales and Net Proceeds of Publications	39 13 8
	£198 1 2

Development Fund Account

To Salaries—		
Naturalist-in-charge	£380 0 0	
Superannuation	21 13 4	
		401 13 4
Curator		200 0 0
Assistants		157 0 0
,, Books, Apparatus, Supplies		208 14 1
,, Ordinary Repairs to Premises		265 10 10
,, Printing, Stationery, Postage		61 5 10
,, Expenses of Naturalist to Lowestoft Conference		8 12 9
,, Balance—Credit to Balance Sheet		58 4 10
(Allocation of Expenditure is subject to revision by the Commissioners).		£1361 1 8

By Balance—1 August, 1921	£439 13 4
,, Grant from Development Fund	921 8 4
	£1361 1 8

APPENDIX C (1)

STUDIES OF PHOTO-SYNTHESIS IN MARINE ALGÆ.

1. RELATIVE PHOTO-SYNTHETIC ACTIVITY OF GREEN, BROWN AND RED SEA-WEEDS IN LIGHT OF VARYING INTENSITY AND FROM DIFFERENT REGIONS OF THE SPECTRUM.
2. VARIATIONS IN REACTION OF THE WATER IN ISOLATED POOLS ON THE SEA-SHORE AS A RESULT OF PHOTO-SYNTHESIS.

By (the late) BENJAMIN MOORE, F.R.S.,
EDWARD WHITLEY, M.A. and T. ARTHUR WEBSTER.
(*From the Marine Biological Station, Port Erin, Isle of Man, and the Department of Bio-chemistry, Oxford University.*)

A number of the experiments recorded below were carried out at Port Erin in the spring of 1919, and it had been intended to publish them along with other work done at the same time on the fixation of atmospheric nitrogen by marine algæ.* Delay, however, ensued in the publication of the results, and as the work was continued in March and April, 1920, it was thought better to hold back the appearance of the account of the earlier experiments until they could be confirmed and extended by further researches.

The figures given as dry weight in Table I were obtained as described in the previous paper, the work being carried out at the National Institute for Medical Research, Mount Vernon, Hampstead.

PART I.

PHOTO-SYNTHETIC ACTIVITY IN LIGHT OF VARYING TOTAL INTENSITY AND IN DIFFERENT COLOURED LIGHTS.

The increased alkalinity produced in sea-water as the carbon of the bicarbonates is organically fixed can be used as a quantitative test for the intensity or velocity of the process in white light of varying intensity, or in coloured lights. This has been done with the results shown in Tables I to VI. Unfortunately, the coloured glass plates, obtainable on the Island at the time, were not spectrally pure, and no means were locally available for comparing the intensity of light which they respectively let through. It is not claimed, therefore, that the figures given of alkalinity developed under the various colour screens can be taken as accurate measurements of the relative photo-synthetic activity of the different regions of the spectrum, but the results show qualitatively that light from widely different zones of the spectrum can produce photo-synthesis, and, secondly, that a large proportion of the total available light-energy can be screened off without materially impeding the process in certain species of algæ.

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* Moore, Whitley and Webster, *Proc. Roy. Soc.*, *B.* 92, 1920, p. 51.

In all cases the optimum daily illumination, as measured by duration and intensity of light, was not found to correspond with the maximum day and sunlight exposure available, but to something much short of this in the case of the red and brown algæ; and even in the case of the green plants there were indications that although these could bear greater illumination yet on a bright day in the period of year concerned (end of March and beginning of April) a certain degree of shading, as behind pale green glass, produced more rapid photo-synthesis than complete exposure. For example, exposures in bottle-glass (green) bottles with lawn covers, often gave an alkalinity half as great again as exposures in open pie-dishes alongside with entirely unshaded action of the full sunlight. This effect varied with the brightness of the day, and was not obtained on dull days.

It is a fact, long well known, that the colours of the different types of algæ vary in an interesting fashion with the depth of their habitation on the littoral, the green flourishing highest, next the brown, and after the red in the greater depths. The subject appears first to have been written upon by Forbes, and later by Oersted, Lorenz, von Berthold, Engelmann, Gaidukov, and others.* Most of the observers have concerned themselves mainly with the adaptation of the colour scheme of the alga to the colour of the light transmitted to that sea-depth at which it flourishes. Thus Engelmann, using his oxygen bacterium and micro-spectroscopic method, shows that the colour of the alga is complemental to that of the incident light. The green algæ near the surface photo-synthesize most rapidly with the red rays, the red algæ growing in the deep "green grottoes" utilise best the green rays complemental to their own red colour. This observer also showed that in the micro-spectrum most oxygen was produced within the red spectral zone (between *B* and *C*) in the case of green algæ, and in the green region in the case of red algæ. This appears to him to show that chlorophyll is not the only pigment related to photo-synthesis; so he generalises this function to the brown and red pigments also, and applies the name chromophylls to the group of photo-synthetic pigments.

The general question of the influence of light of different colours on CO_2 assimilation was worked at by Kniep and Minder.† These authors employed the monocotyledonous plant *Elodea canadensis* and used coloured screens for red and blue light, and a mixture of $K_2\ Cr_2\ O_7$ and $Cu\ SO_4$ in solution for green. They found by the oxygen bubble method that in red and blue light of approximately equal intensity the assimilation was about the same, while even in much stronger green light there was little or no assimilation. Ursprung‡ criticises their conclusions on the ground that the arrangements made by them for the exclusion of the infra-red and ultra-violet rays were inadequate, and that if these were present the total light energy, as measured by the thermopile, under different coloured shades might be the same, while the amount of light of the different wave lengths it was intended to compare might be widely different. Ursprung, himself, projected the spectrum upon the leaves of various green phanerogamous plants which were free from starch—a method first employed by Timiriazeff in 1890. After exposures of various durations the chlorophyll was extracted and the leaf tested for starch by means of iodine. By the use of this method he shows in a series of papers* that while assimilation for green plants is greatest in the region *B* to *C*, yet some assimilation takes place throughout the whole of the visible spectrum, and also in the ultra-violet rays up to $342\mu\mu$ in sunlight, and $330\mu\mu$ with arc lamp illumination. Our results with coloured screens recorded below confirm this view.

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* For key to literature see Engelmann, *Botanische Zeitung*, 1882, 1883, 1888; von Berthold, *Mittheil. aus. d. zool. Stat. zu Neapel*. Bd. 3, 1882.

† Uber den Einfluss verschiedenfarbigen Lichtes auf die Kohlensaüreassimilation. *Zeit. f. Bot.* I, 1909, p. 619.

‡ Uber die Bedeutung der Wellenlänge fur die Stärkebildung *Ber. deut. botan. Gesell.* 1918, XXXVI, p. 86.

TABLE I. *Photo-synthesis by green, brown, and red algæ in sunshine, diffuse light and darkness. April 3 and 5, 1919.*

	Sunshine.	Diffuse.	Dark.
GREEN ALGÆ—			
Enteromorpha compressa	10·6, 9·6	1·8, 1·2	−0·9, −1·4
BROWN ALGÆ—			
Fucus serratus	8·9, 7·5	0·5, −1·1	−1·2, −5·1
Desmarestia aculeata	2·9, 1·0	2·6, 2·6	−0·7, −0·8
Laminaria digitata	4·8	0·7	−4·6
Averages	5·0	1·0	−2·9
RED ALGÆ—			
Plocamium coccineum	1·0	3·4, 2·3	0·9, 1·1
Polysiphonia elongata	3·8	3·8, 3·4	0·0, 0·3
Delesseria sanguinea	2·4, 1·1	3·5, 2·6	−1·2, −1·1
Rhodymenia palmata	6·5	2·1, 4·1	−1·6, −2·5
Averages	3·3	3·2	−0·5

The figures given represent c.c.s. of N/100 acid or alkali (negative sign) required to neutralise 100 c.c. of the sea-water in which the weed had been grown. About ½-gram. of weed was used in each case except in those of Fucus, Laminaria, and Rhodymenia, when somewhat larger quantities were employed so that the amount of photo-synthesis for an equal weight of these three weeds would be smaller than shown by the above figures.

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* *Ber. deut. botan. Gesell.*, 1917 and 1918.

TABLE II. *Photo-synthesis by green and brown algæ in light of various degrees of intensity. 29, 30, 31 March; 1 April, 1920.*

		Material A	Material B	Material C
29.III.	2 hours sunshine	6·0	6·1	3·7
30.III.	29 hours, very little sun	10·5	11·6	9·0
31.III.	23 hours, dull	11·6	10·7	8·7
1.IV.	2½ hours, sun 19½ hours dull	13·0	13·2	9·4
29.III.	Diffused Light 2 hours	2·5	...	2·0
30.III.	Diffused Light 29 hours	1·7	...	0·5
31.III.	Diffused Light 23 hours	0·1	...	−0·7
1.IV.	Diffused Light 22 hours	−0·8	...	−2·0
29.III.	Darkness 2 hours	2·3	...	1·3
30.III.	Darkness 29 hours	−0·4	...	−1·2
31.III.	Darkness 23 hours	−1·3	...	−2·0
1.IV.	Darkness 22 hours	−1·6	...	−2·6
Moist weight at end of Experiment		2·15 (sun)	2·1 (sun)	1·56 (sun)
Moist weight at end of Experiment		2·0 (diff.)	...	1·56 (diff.)
Moist weight at end of Experiment		1·15 (dark)	...	1·54 (dark)
Dry weight at end of Experiment		0·508 (sun)	0·433 (sun)	0·399 (sun)
Dry weight at end of Experiment		0·437 (diff.)	...	0·324 (diff.)
Dry weight at end of Experiment		0·193 (dark)	...	0·298 (dark)

"Material A" is green conferva from the fish-hatching pond.
"Material B" is *Enteromorpha compressa.*
"Material C" is Pylaiella, a brown weed growing on the sides of the fish-hatching pond.

The figures represent c.c.s. of N/100 acid (positive sign) or alkali (negative sign) as in Table I. Two grms. of each weed was taken and weighed after drying lightly between filter papers. 400 c.c. of the sea-water was used in each case. 100 c.c.s was taken daily for the titration and a corresponding volume of water was immediately added.

He also draws attention to the fact that starch formation reaches a maximum after a certain amount of exposure to sunlight, and then declines if the exposure is continued owing, he believes, to damage to the chloroplasts.

So far our own experiments with the three different types of algæ have not concerned themselves with change in colour of the incident light, but with change of intensity of illumination accompanying depth, a factor which seems to us of considerable importance. The results do appear, however, to indicate, as Engelmann urges, that the other pigments, as well as chlorophyll, take an active share in photo-synthesis, as catalysts, and do not merely act as passive colour screens.

Looking at Table I, it might at first be supposed that in the bright sunshine the red algæ synthesized so much less rapidly than the green, because their red pigment screened off the intense light, so saving them from injury, or tending to do so. But this will not serve to explain two facts observed in the diffuse light, first that the red algæ here synthesize twice as rapidly as do the green, and, secondly, that amongst the reds themselves, the rate of synthesis is as great, and in some cases greater, in the diffuse than in the bright light. It does not appear to us possible that any negative screening can explain this, and we conclude with Engelmann that the pigments of the greater depths play a positive part in the synthesis.

The figures taken as a whole show clearly the adaptation to environment, the green plants growing near the surface have their activity greatest in bright light, the reds work best in dimmer light, the browns lie intermediately.

The observations under coloured glass screens were made upon a green alga, *Enteromorpha compressa*, a red (*Ceramium rubrum*) and a brown. The results are not devoid of interest and are given concisely in the following Tables (III to VI).

Six small circular pie-dishes each of about 300 c.c. capacity, were used for the experiment. Each was covered by a slip of glass, except in some instances one of the controls. The colour of the glass slips used is given in the Tables, but it should be noted that the slips employed to give the results in Table III were not the same as those used in subsequent Tables. The Blue in Table III more nearly resembled the Light Blue in the following Tables, and the yellow was intermediate between the yellow and the orange. All were exposed to such sunlight as was available outdoors, and titrated as to developed alkalinity daily. Each dish contained daily 100 c.c. of fresh sea-water and 0·5 gram. of moist weed pressed before weighing in filter paper. The figures given indicate c.c. of centinormal alkali necessary to neutralise 100 c.c. of the sea-water in which the alga had been immersed; the normal figure for sea-water at this period of the year may be taken at about 2·5 c.c. per 100 c.c.

TABLE III. *Photo-synthetic activity under plain and coloured glass slips. 0·5 grms. of green* Enteromorpha compressa. *2, 3, 4, 6, and 7 April, 1919. Alkalinity as represented by c.c.s. of N/100 H.Cl.*

		2.IV.	3.IV.	4.IV.	6.IV.	7.IV.	Average.
Experiment 1.	Clear white glass	7·9	9·8	9·7	9·6	9·7	9·3
,, 2.	Clear white glass	8·7	9·8	11·6	9·5	6·3	9·2
,, 3.	Yellow glass ...	5·0	4·8	7·6	9·8	10·0	7·4
,, 4.	Green glass ...	6·3	8·3	9·0	7·7	7·1	7·7
,, 5.	Blue glass ...	7·6	8·6	6·6	9·6	9·5	8·4
,, 6.	Red glass ...	9·7	9·6	10·6	10·8	11·3	10·4

On 6.IV and 7.IV, Experiment 2, the glass was removed and the alga was exposed directly to the light. It is to be observed that there was no increase in photo-synthetic activity, and that for the most part the dish screened with red glass possessed a distinct advantage. This red glass was a deep red shutting down distinctly the total light intensity. This establishes further by this method of titration of alkalinity the observations by other methods that light from the red region is most effective for photo-synthesis in green cells.

TABLE IV. *Photo-synthetic activity under plain and coloured glass slips. 0·5 grms. of green* Enteromorpha compressa. *1, 3, 4, April, 1920. Alkalinity as represented by c.c.s. of N/100 H.Cl.*

		1.IV.	3.IV.	4.IV.	Average.
Experiment 1.	No cover	10·3	12·0	9·6	10·6
,, 2.	Plain glass	10·3	12·8	8·7	10·6
,, 3.	Yellow glass	10·0	13·7	9·4	11·0
,, 4.	Orange glass	8·7	...	7·3	8·0
,, 5.	Green glass	9·0	10·8	6·7	8·8
,, 6.	Light blue glass ...	10·6	12·5	8·0	10·4
,, 7.	Blue glass	1·7	3·4	3·6	2·9
,, 8.	Deep purple glass ...	1·1	3·5	3·5	2·7

April 1. 5¾ hours exposure, 3 being direct sunshine.
April 3. 2 days' exposure, last afternoon bright sun.
April 4. 2½ hours' exposure to bright sun.

TABLE V. *Photo-synthetic activity under plain and coloured glass slips. 0·5 grms. of red* Ceramium rubrum. *5 and 6 April, 1920. Alkalinity represented by c.c.s. of N/100 H.Cl.*

		5.IV.	5.IV.	6.IV.	Average.
Experiment 1.	No cover.	5·1	4·7	7·4	5·7
,, 2	Plain glass	3·9	2·0	7·5	4·5
,, 3.	Yellow glass	5·5	8·1	9·0	7·5
, 4.	Orange glass	3·7	2·6	5·9	4·1
,, 5.	Green glass	5·5	6·8	8·8	7·0
,, 6.	Light blue glass ...	5·2	4·6	5·8	5·2
,, 7.	Blue glass	1·9	1·9	2·0	1·9
,, 8.	Deep purple glass ...	2·6	2·7	2·5	2·6

April 5. 2.20 p.m. 2 hours' exposure to bright sun.
April 5. 6.0 p.m. 5½ hours' additional exposure, 3½ hours' bright sun.
April 6. 6.45 p.m. 6¾ hours' exposure, 6 hours' bright sun.

Table VI. *Photo-synthetic activity under plain and coloured glass slips. 0·5 grms. of brown* Fucus vesiculosus. *7 April, 1920. Alkalinity represented by c.c.s. of N/100 H.Cl. Number of experiment and illumination as in Tables III-V.*

Experiment	1	2	3	4	5	6	7	8
Alkalinity	2·9	2·7	2·7	2·6	2·1	2·6	...	1·5

6½ hours' exposure on a dull day.

Note.—All the above figures for brown weed are much lower than those for red and green, but while the latter had the advantage of sunshine, the Fucus was only exposed on a dull day.

Commentary on Tables III to VI.

The figures obtained under blue and deep-purple glass are in every case below, and in all except on a sunless day very far below the others. It is possible that this may be partly attributed to the very little light that they let through, for in the case of the purple glass the only visible light was red, which, had it been strong enough, should have given a maximum synthesis with green weed as in Table III. Those under yellow, green and light blue glass show a photo-synthesis comparable to, and in certain isolated instances even exceeding those that were unprotected by a colour-screen.

Spectroscopic Examination of the Coloured Screens Employed.

The screens employed in the year 1919 (results given in Table III) were, unfortunately, not submitted to spectroscopic examination. Those used in the following year gave results as under :—

Yellow. Lets through the red, yellow, and about half the green rays.

Orange. Very similar to above, but the light that passes through is less intense.

Green. No red, faint yellow, strong green, half of blue of longer wave length, no short wave length blue or violet.

Light Blue. Both sides of red cut off—intermediate portion bright. No yellow allowed through, and very little green. No extreme violet.

Blue. Almost opaque—exceedingly faint in blue and green (the blue glass employed in Table III, 1919, was much more transparent).

Deep Purple. Allowed all the red rays through, but only faintly. No rays of any other colour were visible.

PART II.

VARIATIONS IN REACTION OF WATER IN POOLS ON THE SEA-SHORE AS A RESULT OF PHOTO-SYNTHESIS.

In a previous paper* we advanced the views that the development of alkalinity as a result of photo-synthesis in isolated pools must be such as markedly to influence the rapidity of cell-division, and, consequently, of multiplication, and possibly to initiate variations in the organisms inhabiting them. We based our reasoning on the facts (1) that a considerable increase of alkalinity in the sea-water can be demonstrated in the spring, when rapid multiplication begins to take place; (2) that a still greater increase is reached in a more confined space (*e.g.*, the pond used for spawning purposes at the Port Erin Biological Station)† and (3) that when algæ are cultivated in vitro exposed to a good light a degree of alkalinity is attained about equal to that shown by Moore and others‡ to promote rapid and disordered cell-division in the dividing cells of fertilized echinus eggs.

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* Moore, Whitley and Webster, *loc. cit.*

† Moore, Prideaux and G. A. Herdman, "Studies of Certain Photo-synthetic Phenomena in Sea-water." *Trans. Biol. Soc. of Liverpool.* Vol. XXIX, p. 233 (1915).

‡ Moore, Roaf and Whitley, *Proc. Roy. Soc. B.* Vol. LXXVII, p. 102 1905.

It remained to be shown, however, that this extreme degree of hydroxyl-ion concentration could be attained under perfectly natural conditions, and it was with a view to assuring ourselves on this point that the following observations were made.

A number of pools on the sea-shore were selected and samples of water were from time to time collected from each of them in carefully-washed, wide-mouthed glass-stoppered bottles, filled to the top. These samples were then at once removed to the laboratory and titrated. A description of the pools selected follows, and the results of the titrations are given in Table VII, expressed as c.c.'s. of $N/100$ acid required to bring the water to neutrality to phenol-phthalein.

Description of Pools.

No. 1. Good sized pool of no great depth. Chief weed which covered most of the bottom in abundance *Corallina officinalis.*
No. 2. Much smaller pool at a higher level. Plenty of weed nearly all green.
No. 3. A very deep pool with comparatively little weed.
No. 4. A small pool practically free from weed.
No. 5. Similar to 2, but smaller and shallower.
No. 6. A small pool full of Corallina.
No. 7. A pool at a high level poor in weed.
No. 8. Rather poor in weed.
No. 9. Pool specially chosen for its masses of green weed.
No. 10. Connecting with 3 above, but with rather more weed in proportion to the water.
No. 11. Another pool chosen for abundance of green weed.
No. 12. Fair amount of weed.

The relative levels of 1, 3, and 6 were carefully noted. No. 1 was at the lowest level and left by the tide when about one-third of the way out.

No. 3 was uncovered about 25 minutes earlier, and No. 6 two minutes before this. The other pools were all at higher levels, and 2, 4, 5, 7, 8, 11, and 12 were not always reached by the tide.

TABLE VII. *Photo-synthetic activity in isolated shore pools as measured by the alkalinity of the pool water expressed in c.c.s. of N/100 H.Cl. 3 to 9 April, 1920.*

ool No. ...	1	2	3	4	5	6	7	8	9	10	11	12
pril 3 ...	7·2	...	...	...	...	...	...	...	...	...	...	...
,, 4 ...	...	8·4	...	...	...	...	...	...	...	...	...	...
,, 4 ...	6·4 11·2	...	4·7	...	...	...	...	...	...	...	...	...
,, 5 ...	0·3	9·2	0·6	1·2	5·2	0·5	...	...	...	...	...	...
,, 5 ...	5·6	11·3	3·7	2·0	5·6	6·3	...	...	...	...	...	...
,, 6 ...	...	11·8	...	...	6·6	...	4·2	4·2	10·1	5·7	...	...
,, 6 ...	4·9	10·0	3·9	1·0	5·7	...	...	...	10·6	...	...	...
,, 7 ...	...	2·6	1·6	...	5·8	...	...	2·9	6·8	...	...	...
, 7 ...	...	4·8	...	...	4·4	...	...	...	...	...	...	...
,, 8 ...	6·2	...	2·0	...	...	...	...	...	...	...	...	...
,, 8 ...	...	...	...	...	...	...	...	...	10·4	...	10·8	...
,, 8 ...	...	9·6	...	...	9·6	...	...	...	...	...	...	...
,, 9 ...	...	11·9	4·0	...	8·3	8·2	...	8·4	10·6 6·6	...	...	6·6
Averages	6·9	10·5	3·7	1·5	7·2	7·25	4·2	6·3	10·4	5·7	10·8	6·6

The illumination was as follows :—

April 3.	6.0 p.m.	Sunless morning ; bright afternoon ; 3½ hours since pool ebbed.
,, 4.	10.15 a.m.	1½ hours' early morning sun.
,, 4.	6.0 p.m.	Good sunshine most of the day.
,, 5.	7.0 a.m.	
,, 5.	5.30 p.m.	Sunny day till 4.0 p.m.
,, 6.	12.0 noon.	1 hour of bright sun.
,, 6.	6.45 p.m.	Bright sun from 11.0 a.m. to 6.0 p.m.
,, 7.	12.0 noon.	Very dull all day.
,, 7.	3.0 p.m.	High tide ; very dull all day.
,, 8.	12.45 p.m.	Strong sun till 10.30 ; weaker till 1.0 p.m.
,, 8.	2.0 p.m.	No direct sunlight after 1.0 p.m.
,, 8.	3.50 p.m.	No direct sunlight after 1.0 p.m.
,, 9.	2.0 p.m.	Dim sunshine all day.

NOTES :—

1. The reading 6·4, pool 1, April 4, was obtained from a sample taken where the weed was thinnest. 11·2 was obtained from a sample taken where the weed was thickest.
2. Reading 2·6, pool 2, April 7. The pool was not reached by the tide on the preceding days. On April 7 it was entered. The lowness of the reading so caused is accentuated by the dull day.
3. The lower reading, 6·6, pool 9, April 9. The water was titrated just as the tide left the pool. The tide was low and did not reach most of the pools on this day.
4. The averages for pools 1, 2, 3, 4, 5, 6, and 8. The readings at 7.0 a.m. on the 5th, and all readings on the 7th are omitted.
5. The average 10·4 for pool 9. The second reading, 6·6, pool 9, April 9, and the reading 6·8, pool 9, April 7 (no sun), are omitted.

Commentary on Table VII.

NOTE (1). The high level of alkalinity attained to in pools rich in green weed—Nos. 2, 5, 9, and 11 ; 5 is not as high as the others, but the volume of water in it was very small, and a little fresh water in the form of rain would, therefore, make a considerable difference to the result.

NOTE (2). That the coralline pools, Nos. 1 and 6, are some distance behind the green ones in alkalinity, but still attain to a fair level. The green pools lying higher on the beach had a longer separation from the tide. This gave them more time to photosynthesize and, doubtless, accentuated the difference.

NOTE. (3) That those pools which are poor in weed relatively to the volume of water, Nos. 3, 4, and 7, do very little photo-synthesis.

NOTE (4). The low level of alkalinity when the titrations were done in the early morning before the sun had been shining on the water and on the day when there was no sunshine.

The above observations show conclusively that processes are at work in nature similar to those that have been demonstrated when algæ are grown under artificial conditions in the laboratory. In every pool where there is a fair supply of weed a hydroxyl-ion concentration is reached far surpassing that found in the open sea or even in the confined waters of a bay. A difference in the reaction of the water such as has been shown to exist cannot but exercise a profound influence on the organisms exposed to it, and must form one of the many factors tending to limit the number of species which can successfully colonize the sea-shore.

SUMMARY.

1. Comparative experiments with green, brown, and red algæ show that these are arranged upon the littoral so that each kind is in that intensity of illumination which is the optimum for the colour scheme of chromophylls it possesses. In strong illumination green algæ synthesize far more rapidly than red, but in weak illumination the red algæ synthesize more rapidly than the green. Furthermore, the reds for the most part synthesize more rapidly in weak light than they do when exposed to direct sunshine. The browns are intermediate in their action between the greens and the reds.

It follows that the effect of the red pigment is not that of a negative screen, but that like the green chlorophyll it possesses a positive catalytic action in photo-synthesis.

2. By a method which the authors believe to be novel for this purpose, viz.: the titration to phenol-phthalein of the alkalinity developed in the fluid in which the weeds are grown—it is shown that light from widely separated regions of the spectrum can be photo-synthetically active, the observation by other methods that the red rays are most effective for this purpose being confirmed.

3. A comparative study of the relative alkalinity obtaining in various isolated salt-water pools has shown that, under favourable conditions, as high a concentration of hydroxyl-ions is reached there as in experiments in vitro, thus affording strong experimental support to the views advanced in a previous paper as to the part that may be played in the annual spring development of life along our coasts by the increasing alkalinity of the water.

The expenses of the expedition to Port Erin and of the materials used there were defrayed by a grant from the Percy Sladen Memorial Trust, those for materials in London from the funds of the Medical Research Committee; and our thanks are due to the Trustees and Committee for this valuable support. We are also indebted to Professor Sir W. A. Herdman, F.R.S., for much valuable advice and assistance.

Appendix C (2).

FOOD OF YOUNG PLAICE AND PLANKTON OF THE SPAWNING POND IN 1922.

By Andrew Scott.

(With a Note by J. R. Bruce.)

Tow-nettings were taken from the spawning pond once a week throughout 1922, so that some idea could be obtained of the quantity and variety of the organisms therein. The samples were sent to me for examination and report. It is not intended here to give a detailed statement of the results. That will probably appear elsewhere later on. A summary of the plankton prevailing in the first six months of the year, a time of vital importance in the rearing of young plaice, may be useful, however, at this stage.

A good deal of work has already been done to determine the plankton in the pond and also the food contents in the stomachs of larval and post-larval plaice hatching from the eggs spawned by the adults and developing naturally in it.

In 1921 I examined the stomachs of just over 800 of these young stages, which had been preserved in weak formalin. Sir William Herdman, about the same time, was examining living specimens on the spot, and his manuscript notes were sent to me so that our results could be compared. My results were published in the "Journal of the Marine Biological Association," in October, 1922,* and Sir William's have been published in "Spolia Runiana" V, Jour. Linn. Soc., July, 1922, p. 168. He was satisfied that the plaice larvae begin to feed by taking solid food through the mouth long before the yolk-sac is exhausted. He also found that the food contents consisted of algal spores, diatoms and exoskeletons of copepoda. None of my larval stages indicated attempts to catch external food while any of the yolk remained, nor could I find any diatoms amongst the food consumed by the post-larval stages. This, no doubt, shows the value of examining living material whenever it is possible, instead of relying wholly on the results one obtains from preserved specimens. There is probably a certain amount of regurgitation of the stomach contents of these delicate stages which is brought about by the action of the preservative.

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* Vol. XII, No. 4.

Dr. Marie Lebour, who has made extensive investigations of the food contents of the stomachs of a large number of young fishes, states: " Fish caught in the act of swallowing copepoda always show the tail sticking out of the mouth, so that they are swallowed head first." I noticed in some of my specimens that had fed on copepoda (Harpacticoida) that the copepods had apparently passed rather rapidly through the alimentary system and reached the rectum undigested. In these instances the tail setae of the copepods were always projecting through the anus to the exterior. In fact, the copepods seemed so perfect and apparently undigested that one would almost be justified in concluding that they had entered through the anus and not through the mouth! It is just possible, however, that the copepods were feeding on the young plaice and had entered by the most accessible opening. Some of the ostracoda are known to feed on adult fishes which have been left too long on the hooks after being caught. Professor G. O. Sars, in the first part of his new volume on the ostracoda (" An Account of the Crustacea of Norway"), in describing the habits of *Cypridina norvegica*, Baird, one of the semi-pelagic species, states: " It is pronouncedly rapacious, feeding eagerly upon any carcass found on its way, and in some instances even attacking living animals. Dr. Ostergreen found on one occasion several still living specimens of this Ostracod within the cloaca and the uterine cavity of a *Spinax* *niger* caught in the Trondhjem Fjord and was thereby led to the supposition that this form leads a parasitic life in the said fish. This is, however, by no means the case. The Ostracods had simply attacked the *Spinax* when hanging on the fishing-line in a dead or feeble condition, and had entered the most accessible openings of the fish in order to feed upon its soft parts. The finding of allied Ostracods on the gills or other places on recently captured fishes, as mentioned by some other authors, may be explained in a similar manner."

The samples of plankton represented the results of drags of about fifteen minutes each with coarse and fine nets. The material was examined quantitively just in the same way as the plankton collected during the "Intensive Study Investigation" of the open sea was dealt with. The volume of the combined catch varied from 1·3 to 8·9 cubic centimetres, and reached its maximum during the time the plaice were spawning. It was then largely made up of eggs. The examination of the samples showed that plaice eggs first appeared amongst the plankton of the pond on February 9th, and continued with two exceptions, March 23rd and 28th (when my samples contained no eggs or larvae), to May 2nd. The largest number of plaice eggs captured was 1,400. That was on March 14th. Plaice larvae were first observed on March 7th. They were irregular in occurrence and the numbers varied from one to thirteen. No larvae were found after the middle of May. Other fish eggs, chiefly gadoids, such as cod and pollack, were also found from time to time. Probably they had been drawn in through the pumps along with the water, but their numbers at times were rather surprising. Pollack eggs were first noted on February 9th and lasted for about a month. They reached a maximum of 177 on February 23rd, but were no longer present after April 10th. Cod eggs had a duration of apparently only eight days from February 23rd to April 3rd. 1,049 cod eggs were collected by the two nets on February 23rd, but after that date the numbers varied from two to seventeen. It is well known that there may be a very considerable difference in the sizes of the eggs spawned by the same fish, and it is not always possible to separate some of the species of gadoids, for instance. Plaice eggs, no matter how much they differ in size, have a distinctly striated shell and are identified easily. The characteristic larvae of cod appeared in the plankton of the pond on March 14th and April 3rd. The plankton organisms occurring in the pond which form the food of the larval and post-larval plaice are, no doubt, largely drawn in along with the water from the sea, and as the plankton increases in the sea so does that in the pond. Some of the copepoda belonging to the Harpacticoida, such as *Idyaea*, are practically permanently established and one generation succeeds another. A species of rotifer, *Notholca striata*, O.F.M., is also seldom absent from the pond plankton. During the whole of January *Idyaea furcata* and its nauplius was the most abundant form of food. On January 14th the catch was almost entirely this copepod alone. The adults numbered 11,000, of which 1,500 were females with egg sacs. There were also 7,500 nauplii of *Idyaea*. The only other organisms on this date were 100 barnacle nauplii and 110 *Biddulphia mobiliensis*. A week later there were ten different kinds of organisms. The numbers increased each succeeding week, and by April 10th, just about the time the plaice larvae were appearing, there were twenty-four different kinds of organisms, exclusive of fish eggs and larvae. Barnacle nauplii were represented by 4,220, *Chaetoceras decipiens* 5,820, and *Coscinodiscus radiatus* 2,100. The maximum was reached on April 26th. The most abundant organisms were barnacle nauplii 14,000, *Chaetoceras debile* 14,400 *C. decipiens* 9,680, *C. teres* 6,800, and *Notholca* 7,400. The plankton in the pond on June 5th was nearly pure *Podon*. The estimation gave 22,772 individuals. If one takes into account the many very small plankton organisms such as spores of algae, etc., that pass through the nets, and the larger forms that the nets retained, I think it can be concluded that there was enough plankton in the pond to supply the young plaice hatched in it in 1922 with food during their pelagic life.

The following is the list of organisms found in the plankton of the pond between January and the end of June :—

DIATOMS—

Asterionella bleakeleyi
Biddulphia mobiliensis
,, *sinensis*
Chaetoceras debile
,, *decipiens*
,, *teres*
Coscinodiscus concinnus
,, *radiatus*
Coscinosira polychorda
Ditylium brightwellii
Guinardia flaccida
Lauderia borealis
Streptotheca thamensis
Thalassiosira gravida
,, *nordenskioldii*
Rhizosolenia shrubsolei
,, *stolterfothii*

PERIDINIANS—

Ceratium tripos
,, *furca*
,, *fusus*
Peridinium spp.

COELENTERATA—

Medusoid gonophores

VERMES—

Sagitta bipunctata
Polychaeta larvae
Notholca striata
" Mitraria "

CRUSTACEA—

" Zoea "
" Mysis of Crangon "
Podon intermedium
Evadne nordmanni
Calanus septentrionalis
Pseudocalanus elongatus
Temora longicornis
Centropages hamatus
Acartia clausi
Oithona similis
Amphiascus minutus
Dactylopusia tisboides
Idyaea furcata
,, *gracilis*
Laophonte stromi
,, *proxima*
Mesochra pygmaea
Tigriopus fulvus
Westwoodia minuta
,, *monensis*
Nauplii of Copepoda
,, ,, Barnacles
" Cypris " of Barnacles

MOLLUSCA—

Pelagic larvae of Gasteropoda
,, ,, ,, Lamellibranchiata

TUNICATA—

Oikopleura flabellum

FISHES—

Plaice eggs and larvae
Cod eggs and larvae
Pollack eggs
Whiting eggs
Rockling eggs
Flounder eggs

(*Note to Mr. Scott's paper*).

The occurrence of eggs, other than those of the Plaice, in the Plankton of the spawning ponds is to be accounted for, at any rate in part, by their escape, with the outflowing water, from the aquarium in which a number of gadoid fish (cod, whiting and pollack) were observed to be spawning. To settle this point the aquarium outflow was caused to pass through a canvas bag before falling into the spawning ponds, and on March 6th and again on March 10th large numbers (110 c.c. in all) of eggs were found in the bag. Some of these were placed in the hatching boxes, but they died and settled on the bottom after a few days, being unfertilised.

On another occasion a few larvae were reared from eggs similarly collected, but the species was not identified.

The ephyrae of *Aurelia aurita* and *? Cyanea* frequently observed in former years, were noted in considerable abundance, about February 21st.

J. R. Bruce.

APPENDIX C (3).

OTHER INVESTIGATIONS MADE IN THE DEPARTMENT OF OCEANOGRAPHY.

The following papers on Marine biological subjects were published by the Department during the year 1922. The references (unless otherwise stated) are to the "30th Annual Report of the Lancashire Sea-Fisheries Laboratory at the University of Liverpool and the Sea Fish Hatchery at Piel" (Liverpool, April, 1922).

(1) W. Birtwistle and H. Mabel Lewis.
"A Biometric Study of Irish Sea Herrings."
(pp. 180-204.)

(2) W. Birtwistle.
"Report on the Mussels in the Ribble Estuary."
(*Supt.'s Report to Lancs. and West. Sea-Fish. Joint Committee*, 31 Dec., 1921, pp. 27-37. Preston, 1922.)

(3) R. J. Daniel.
"Chemical Composition of the Mussel. Tables of Results."
(pp. 205-221.)

(4) Sir W. A. Herdman, C.B.E., F.R.S.
"Spolia Runiana," V. Some Results of Plankton Investigation in the Irish Sea."
(*Journal Linnean Society*, Botany, Vol. 46, pp. 141-170. Pl. 7. July, 1922.)

(5) J. Johnstone, W. Birtwistle and W. C. Smith.
"The Plaice Fisheries of the Irish Sea." (pp. 37-179.)

(6) J. Johnstone.
"Some Diseases and Parasites of Fishes." (pp. 222-237.)

(Papers (1) and (5) relate to work done under the scheme of the Ministry of Agriculture and Fisheries for directed fishery research in the Irish Sea.)

REPORT ON THE INVESTIGATIONS CARRIED ON IN 1922 IN CONNECTION WITH THE LANCASHIRE SEA-FISHERIES LABORATORY AT THE UNIVERSITY OF LIVERPOOL, AND THE SEA-FISH HATCHERY AT PIEL, NEAR BARROW.

EDITED BY

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INTRODUCTION.

In addition to the subjects of research mentioned above some other investigations have been in progress but either the results are held back in the meantime, or they are published elsewhere. A long series of investigations carried out on the Plankton of Port Erin and adjacent areas has now been completed. This work was done by Professor Sir W. Herdman and Mr. A. Scott, and it includes the detailed examination of over 6,000 samples. Each of these has been investigated by Mr. Scott, and the numbers of each of about 30 different species occurring in *each* sample have been estimated. The results were recorded in Schedules which have been stored for future reference (along with the samples themselves) at the

Oceanography Department in the University of Liverpool. A tabulation of the principal results, including fourteen years' investigations, has now been made, and it is expected that these figures, with a discussion, will be published this year, as a separate book. It is hoped that these results will be noteworthy. They represent a vast amount of very patient research done by Mr. Scott, and they probably constitute the most complete systematic investigation of the plankton of a small sea area that has yet been made in this country.

Some additional work on the plankton of the southern part of St. George's Channel has also been done by Mr. Scott, and a study of the plankton of the spawning pond at the Port Erin Hatchery, in relation to the food of the little plaice reared there, has also been undertaken by him. These results are not published, as they are sure to be supplemented by later investigations. Since the last Report appeared, a paper by Mr. Scott has been published in the Report of the Marine Biological Association: this deals with the organisms that are used as food by little plaice that have just completed their transformation and are settling down on the sea bottom.

The work of the Station at Piel has been greatly interrupted during the last year and a half, and it is hoped that more stable conditions may now follow those of a very troubled period. Dr. Jenkins (who is a member of the Advisory Board of Oceanography at Liverpool University) and I have been considering the future of the establishment and we wish the Committee to allow it to be used for the instruction of University students. It is probable that there will be much demand for work-places at Port Erin Biological Station in the future, and even now the accommodation there is very severely strained during the Easter Vacation period. Expansion is impossible (in the near future, at all events) and it is desired that suitable students be sent to Piel, if it should be necessary, under the charge of their instructors.

The Oceanography Department gratefully acknowledges the assistance given by the Joint Committee in allowing Mr. B. Sundara Raj, M.A., Assistant Director of Fisheries for the Presidency of Madras, to work at Piel on plankton research under the direction of Mr. Scott.

Mr. Scott gives the usual short account of the work done at Piel. In this Introduction I add here a summary (for the benefit of non-technical readers) of the main results of the investigations that are dealt with in detail later on in the Report.

GENERAL ACCOUNT OF THE WORK.

The Seasonal Physiology of the Common Mussel.

Particular attention should be given to the paper, by Mr. Daniel, on the changes that occur, during the year, in the bodily structure and the chemical composition of the mussel. Sometime during the spring or early summer the animal spawns and the change in its appearance, when this happens, is so extraordinary that every fisherman has been impressed with it: the mussel, they say, "loses its fish." That is, the edible, soft parts, become so greatly reduced in volume that the mollusc is, for a time, almost valueless as an article of food. The body becomes thin and emaciated and the thick, fleshy flaps (the mantle lobes) that lie close underneath the shell become reduced to thin membranes. This change may occur quite rapidly—even in the bags containing the mussels after they have been gathered and are on their way to the markets. The shells then become covered with a sticky semi-liquid mass which completely spoils the appearance of the product. The mussels have spawned, and the emaciation of their flesh is due to the loss of the genital products (ova and spermatozoa). In the higher animals these products are a very insignificant fraction of the total mass of the organism, but in the mussel (as in the roes of such fish as the herring or cod) they may amount to a very large part of the total weight of the unspawned individual. After the reproductive act the fish, or mussel, is, for a certain period, " out of condition," and far less valuable, as an article of food, than it was in the unspawned condition.

The recovery from this " out of condition " state takes some time in the cod or herring, but it proceeds fairly rapidly in the case of the mussel. After spawning everything is favourable: the temperature of the sea is rapidly rising and food organisms, in the shape of diatoms, the spores of seaweeds, etc., are abundant. The mussel, therefore, feeds greedily and its wasted tissues are rapidly repaired. It grows in size and at the same time the materials necessary for the formation of next year's crop of ova and spermatozoa are accumulated. The ovaries and testes grow rapidly at the expense of this accumulated material. From the eatable point of view the mussel, some little time before it spawns, is in the best condition as an article of food.

The same kind of series of changes occurs in the case of a fish. The flesh, in a herring, becomes loaded with fat and this also accumulates in the peritoneum. In a cod the flesh contains practically no fat, but the latter substance becomes stored in the liver. When spawning has occurred much of this stored fat has disappeared. So far, we are not quite sure as to what is the significance of the stored fat in the flesh or the liver of a fish—whether it is a kind of residue that results from the growth of the roes and milts or whether it is material that is used in the development of the latter organs. But the condition of the fish, as an article of food, runs parallel with the amount of fat that is stored up—the fat-rich herring, before it is quite ready to spawn, is very good to eat, while the fat-poor, spent herring is just as unsuitable as an article of food.

The fat that is contained in the " blubber " lying beneath the skin of a herring, or in the solid substance deposited on the peritoneum, or in the liver of a cod, is not particularly valuable, *in itself*, as food. One loses most of this fat when a herring is cooked, and of course one does not usually eat the liver of a cod, or skate, or ray. But the case is different with the stored material in the flesh of a shell-fish, such as the mussel or oyster. There the fat is different in its composition : it is not "fishy" in flavour, and it is diffused throughout the flesh. Further, the flesh of the molluscan shellfish differs very notably in chemical composition from that of a fish : it contains the food substances called *carbohydrates* ("starchy" or "sugary" compounds) and these are eminently digestible and nutritive. Because of their presence, and the general balance of them with the watery and nitrogenous constituents, the substance of oysters rather resembles rich milk in respect of its dietetic quality. The substance of mussels, at their best, is even better from the nutritive point of view. These carbohydrates are characteristic of the vegetable, rather than of the animal organisms. They are practically absent in the flesh of fishes, but are present in notable proportion in that of the molluscs. This is because of the fact that the latter animals feed largely upon the diatoms—which are microscopic plants.

In two papers, published in the last two Reports, Mr. Daniel has given an account of the changes that occur, from month to month throughout the year, in the chemical composition of the mussel. In the paper now presented he has gone further and has undertaken the very difficult work of tracing out exactly where, in the tissues of the body, these fats and carbohydrates are stored. This has necessitated a study of the ways in which the substances called "fats" and "glycogen" are deposited in the tissues. So far, our knowledge of the chemical nature of fats and glycogen has been based on the investigation of the warm-blooded animals, and it is certain that, when we deal with marine molluscs, such as the mussel and oyster, the conditions are very different. By "fats" we must understand a number of different chemical compounds all having the same *general* character but differing considerably in detail. Glycogen is "animal starch" but, again, it is certainly the case that it is not the same thing in a mollusc as it is in, say, a sheep. The further study of the precise nature of both fat and glycogen in marine fishes and molluscs plainly means very difficult research—which must now be left to the bio-chemists. Here Mr. Daniel traces out, in a very interesting way, the series of visible changes by which the mussel first stores up fat and glycogen in its tissues, laying these substances down as "reserves." Then, when it is preparing for reproduction it draws on these reserves, using the glycogen, in particular, as the source of material from which it builds up its genital products. Obviously many interesting questions are suggested for further investigation by specialised methods.

The Mussel as a Source of Food-stuff.

In the mussels and cockles that inhabit the foreshores of these islands we have an astonishingly abundant source of material which approximates, in its chemical composition, to that of a "perfect food-stuff," the proteid, fat, carbohydrate, and water, having the proportions that are most desirable. Hitherto this supply of food has hardly been exploited and, at the present time, the growing pollution of the shore and the inability of the public authorities to deal with this question in an adequate manner has, still further, reduced the demands made on the shellfish supply. It is now obvious that the development of the shellfisheries as an abundant source of animal food is incompatible with the protection of the public health, on the one hand, and with modern industrial development, on the other—that is to say, it is impracticable to attempt to deal with existing pollution in such a way as to avoid fouling the shellfish beds except at such a cost that would be regarded as impossible. In these circumstances the future utilisation of the mussel supplies probably depends on methods of preservation, such that all pollution dangers would be prevented. The practice of such conservation methods will be facilitated by a study of the chemistry of the crude natural product from the food point of view. It may also be pointed out that this investigation indicates an abundant source of the somewhat rare material, glycogen, and that this may very possibly have important uses in the future.

Investigations on the Cod.

Some investigations into the life-history of the cod, made during the last two years, are summarised in the Report. This was, in part, work done in order to get data that would help in the general study of rhythmic changes in marine animals, and it was also a contribution to the general scheme of research work on the life-history of the cod, that was organised by the Ministry of Agriculture and Fisheries.

We have shown, in former reports, that there is a very marked series of changes in the chemical composition of the flesh of herrings taken in the Irish Sea at different seasons throughout the year: this applies not only to the Manx summer fishery but also to the back-end and winter fishery that occurs off the coasts of North Wales. In the case of the Manx fishery the cycle of changes is as follows:

> Herrings may be taken in March (or even earlier) but they are of very poor quality. The fish are thin; the flesh is watery, and there is little fat beneath the skin. At this time of the year the temperature of the sea is about at its lowest point.
>
> About June the typical summer fishery begins. The herrings rapidly improve in condition. The flesh is firm and there is an increasing percentage of oil in it. The fish

are rapidly maturing. The temperature of the sea is rapidly rising.

About August or September the roes and milts of the herrings become mature. The fish spawn. Just before they spawn, their condition begins to deteriorate so that the percentage of water in the flesh increases and that of fat decreases. The temperature of the sea is beginning to fall.

After this the condition of the spent fish falls off very rapidly. But there is great difficulty in tracing the further life-history of the spent herrings.

It would appear, then, that the annual series of changes in the fish are regulated by the changes in the temperature of the sea. When this is very low (in February and March) the herrings are in very poor condition. In April and May the temperature rises quickly, and in June and July the copepods and other micro-crustacea (upon which the herrings feed) increase very greatly in abundance. During this period of plentiful food the fish put on flesh and their reproductive organs develop. Then, as the temperature falls in September and October, the condition of the herrings deteriorates.

But this cannot be all, for much the same series of changes also occurs in the case of the Welsh winter spawning herrings. Here also the percentage of fat in the flesh increases during the month or two before spawning occurs, but this is just the time of year when the sea temperature is falling most rapidly. Therefore there is some cause for the variations in condition other than a mere rise of sea temperature. The fact is that the processes of putting on flesh and then spawning depend also on something in the organisation of the fish itself, as well as on the physical changes that occur in the sea.

We thought that a very similar series of changes would be observed in the case of the cod. It is well-known that there is a seasonal change in the percentage of oil contained in the livers of the cod taken in the great Norwegian fisheries, and this was also expected in the fish that are caught round the Isle of Man. There are also quite remarkable differences in the oiliness of cod livers in different years, and this can be seen in the statistics of the Norwegian cod fisheries. It was even supposed that these differences (between a good cod liver oil year and a bad one) could be traced back to differences in the temperature and other physical characters of the drift of relatively warm and dense Atlantic water that flows north along the west coast of Norway. In fact, a rough correspondence between the rate of northerly drift of the Atlantic water, from year to year, and the variations, from year to year, in the average number of spots that occur on the sun's face, was said to exist. This, however, is probably a fallacy, but it is quite certain that the condition of the cod does depend, in the long run, upon the temperature and density of the sea water. The association may be an indirect one in this way : the variations in physical conditions in the sea must affect the rate of reproduction of the vegetable plankton and this, in turn, regulates the abundance of the crustacea on which the cod feed. There are certainly big causes at work in the sea affecting the conditions of growth in the case of fish. Thus the end of 1921 and the beginning of 1922 were bad years for the cod and herrings in the North Sea. For some reason or other, the fish were very ill-nourished during those seasons and a fair number of cod were seen with ulcers in their flesh.

The cod that are caught in the Irish Sea are mainly taken round the Isle of Man, over to the coast of Cumberland and down the Lancashire coast towards Blackpool ; elsewhere the fish are not abundant. The fish come into these regions in order to spawn, and they probably come from the North (just as the hake and mackerel come from the South). After spawning the survivors of the shoals probably retrace their migration paths and go out from the Irish Sea. Small, immature cod are, of course, in this area from the time when they were spawned until they become mature, when they probably move out from the Irish Sea, returning again in the winter months to spawn. We hoped, then, to find some definite seasonal changes in (1) the percentage of water in the flesh, (2) in the percentages of water and oil in the liver. and (3) in the percentage of nitrogen in both the flesh and liver. Samples of the larger cod caught near Port Erin were therefore obtained, fortnight by fortnight, during part of 1921, 1922, and part of 1923. It was expected that such changes as were observable in both the herring and plaice would also be seen in the cod. For this reason, approximate chemical analyses were made on the samples received.

These (and some other) numerical results are given in the report by Messrs. Johnstone, Smith, and Fleming. It must be admitted that the results are not as clear as we expected. The seasonal change in condition of the fish—so well seen in the plaice—and in the percentage of fat in the tissues—so well marked in the herring—are not at all evident in the case of the cod. What does seem, however, to be certain is that the cod of 1921 and the first five months of 1922 were very exceptional in some respects. During all that time the percentage of nitrogen contained in the dried tissue of the fish was lower than the average ; it was about 14 to 15 in the case of the dried flesh as against 15 to 16 in the later months of 1922. The percentage of nitrogen in the dried liver (from which the fat had previously been extracted) was about 11 to 12 prior to May, 1922, as against about 17 to 18 in the later months of 1922. As regards nutrition, then, the end of 1921 and the first four months of 1922 were distinctly unfavourable.

The facts that cod in the Irish Sea do not exhibit that very regular change in condition that we see in the herring, and that the fish is not nearly so good an article of food as it is in the North Sea, is attributed to the situation of the former fishing-grounds. The northerly seas are the typical home of this fish. In the Irish Sea it is near its southern limits of distribution and the physical conditions that are most favourable to its growth and nutrition are not displayed by our sea area.

Investigations on the Herring.

Mr. W. Birtwistle and Miss H. M. Lewis have made a further contribution to the work which they have been doing on the life-history of the Irish Sea herrings. This is also part of the scheme of directed research organised by the Ministry of Agriculture and Fisheries. Several reports have already been published and a good deal of work has been recorded on the bio-chemistry of the Manx summer herrings.

The problem to be studied is the origin and migration paths and composition of the shoals of herrings that make the Irish Sea fisheries. Prior to the last two years there were only two fisheries. (1) The summer one that begins about May in Manx waters and ends about September. This fishery depends mainly on herrings that are shoaling for the purpose of spawning. (2) The Welsh fishery: this begins about the southern part of Cardigan Bay somewhere about October and usually ends in Anglesey waters sometime in December or January. These herrings are also shoaling in order to spawn. Where do the fish come from? Where do they go when the shoals disperse? Where exactly do they deposit their spawn? What are the predominant ages of the fish and do these ages vary from year to year? These are the questions to be answered.

The above statements do not exhaust the matter. Large quantities of herrings have been caught during the autumn and winter months by steam trawlers, both in the south and north entrances to the Irish Sea. Herrings may be caught by drifters near the Isle of Man as early in the season as March. Further, herrings of a different kind from those taken near the Island are caught in the off-shore waters. Above all, the last two winters (1921-22 and 1922-23) were very remarkable in that herrings appeared all along the west coast from Great Orme's Head to the Solway Firth and the fish had not been in these waters in quantity for the last thirty years. This reappearance of the shoals was so remarkable as to give rise everywhere to keen interest.

It was therefore desirable to examine the records of past local herring fisheries. It must be admitted that the materials for this search are by no means abundant. *In the interest of future fishery research no pains should be spared to record the occurrence and progress of present fisheries, especially if there is anything unusual.* An historical account of the herring fisheries of the Irish Sea during the eighteenth and nineteenth centuries has been prepared, from such sources of information as are accessible to us, by Mr. W. C. Smith : this account is being published elsewhere. Unfortunately the data that might enable us to associate the remarkable changes of the last two winters with the physical conditions in the sea do not exist. The hydrographic work has not been resumed since 1914.

Mr. Birtwistle and Miss Lewis contribute some further data, mainly referring to the composition of the herring shoals with respect to the age of the fish. In this work they have relied upon the methods of scale and otolith "readings." It is well known that the materials constituting the scales and earstones of herrings, and other fishes, are not laid down regularly. There are differences in the nature of these materials that enable us to say what parts were deposited in the summer and what in the winter : thus the age of the fish is recorded in its scales, earstones, and even in the bones.

The method is admitted to be valid in the case of the scales of the salmon, cod, and some other fishes, and it is also valid in the case of the earstones of the plaice. There has, however, been much discussion as to whether or not it can be trusted in the case of the scales of the herring, and this point is discussed in the paper that appears in the following pages. Certainly experimental work on the processes of scale and earstone formation, and actual observations on fish kept in captivity, are urgently required. Even then much more abundant data than we have hitherto had, in the case of the Irish Sea herring fisheries, is also required and an effort is being made to obtain this during the present summer. Also we await the winter of 1923-24 with much interest in order to see whether or not the remarkable recurrence of the west coast fishery will again be observed.

Malignant Disease in Fishes.

Several additional cases of the occurrence of malignant tumours in fishes are recorded in the following pages: for the specimens illustrating the conditions, and for very careful accounts of the places of capture, etc., we are indebted to some of the Fishery Officers of the Ministry of Agriculture and Fisheries, and grateful acknowledgment is here made.

It may be useful to state, for the benefit of the non-technical reader, what are the general results of the studies of pathological conditions in fishes that have been made in this laboratory for some years past. These conditions are due to (1) the invasion of the outer surfaces (the skin and gills) of the fish, or its cavities (the nostrils, mouth, stomach, intestine, and body cavity), or the flesh itself by parasites of some kind or other; and (2) to " disease " in the ordinary sense of the term. The parasites that are found in fishes are :—

Copepods (*Fish-lice*) present on the outer surfaces, in the mouth, nostrils, gill cavities, etc.

Leeches and Trematodes (*Flukes*) having just the same modes of occurrence.

Cestodes and Trematodes (*Tapeworms and Flukes*) that are found in the stomach, intestine, gall-bladder, body-cavity, or even in the flesh.

Nematodes and Echinorhynchi (*Round Worms*), which occur in the same situations as the tapeworms and flukes.

Protozoa (unicellular organisms), which may be found almost anywhere—in the skin, gills, eyes, brain, on the nerves, in the skeleton (bones and gristle), in the intestine and other sections of the alimentary canal, in the liver, flesh, etc. All these modes of occurrence have been observed.

These are the principal kinds of parasites that have been found in fish hosts. The nematodes (round worms) are still imperfectly known* and much has to be done with respect to the occurrence of bacteria in the tissues and cavities of fishes. (The latter kind of work is, however, more intimately related to the study of fish preservation ; it is being followed up by the Food Investigation Board).

The zoological interest of these investigations is very great, and it was from this point of view that they were first undertaken. More interesting still, however, is the study of the inter-action between the parasite and the host and the investigation of the remarkable way in which the bodily structure and habits of an animal become modified when it adopts a parasitic habit. The alterations in the bodily structure and modes of functioning of a host that is heavily "parasitised" are equally interesting and lead us on directly to the study of a special department of animal pathology.

The effect of the parasite on the host.

This may be very slight—or it may be profound. The ordinary cases of infection by fish-lice do not appear to give the host much inconvenience, and even a fairly large number of external trematodes and leeches may be carried by a fish without apparent ill-effect. A fair proportion of sprats taken in local waters harbour a Copepod parasite about an inch in length: this has anchoring hooks that are immovably fixed *in the eye* of the fish. Many whitings harbour another species of large copepod which has its anchor-hooks embedded in the ventral aorta (the large blood-vessel coming directly from the heart. In the two latter cases the parasites feed by sucking and digesting the fluids (blood, lymph, etc.) of the body of the host, yet it is often difficult to be sure that fishes so attacked are any less healthy than are unaffected ones. The anchored copepods that occur on fishes look like particularly ferocious parasites but, in general, they are no more detrimental to their hosts than are fleas to a dog.

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* This subject is now being studied by Mr. T. Southwell of the School of Tropical Medicine in the University.

Even the tapeworms do not appear to be very malevolent in their effects. In man, or in the domestic animals, a heavy Cestode infection may be very serious in its consequences on the general condition of health, but this is not usually the case in fishes. It is very rare indeed to find a brill or turbot in the Irish Sea which is not heavily infested by large tapeworms (*Bothriocephalus punctatus*) even to the extent that many dozens of worms, each about one to two feet long, may be present, almost blocking up the intestine where it issues from the stomach. Yet such fish may be well-nourished and, so far as one can see, perfectly healthy. This is also the case with skates, rays and dogfishes which nearly always have tapeworms, though not to the same extent as have brill and turbot.

The mere association of host and parasite may, indeed, be considered as almost normal and unprejudicial to either animal in many cases. Such cases, among the fishes, are when the parasites are tapeworms living, in the adult state, in the intestine, or Copepods living on the surface of the body. The same kinds of associations are far more deadly in the case of a warm-blooded animal.

Neither have the other external parasites—the Leeches or external Trematodes—any apparently bad effects on the hosts. Rays have, frequently, large leeches about six inches long, attached to the skin so that there are very obvious wounds when the parasite is forcibly removed : yet the fish itself may be perfectly healthy.

What is far more serious, in the case of a fish, is the occurrence of a parasite *in the flesh itself.* Tapeworms have, each of them, two stages in their life-histories : in the first, or larval stage, the parasite often inhabits the tissues of some animal which is then eaten by the fish. When this occurs the larvae may not be destroyed by the digestive juices that dissolve the flesh in which they are contained, but may then complete their life-career in the intestine of the animal which eats them, living there as fully developed worms which regularly reproduce and carry on the cycle. Dabs, small plaice, etc., may carry in their flesh very numerous tapeworm larvae, or even fully-developed flukes, to such an extent that they appear to be spotted, or " measly." When this is the case the fish is usually much emaciated, the flesh is thin and watery and the general " condition " is thoroughly bad. What happens in such infections is probably the liberation of poisonous substances, or toxins, from the encysted tapeworm or trematode larvae and these produce general functional disorders in the host.

So also heavy infections with protozoan parasites may be particularly destructive. These are rare. Cases, however, occur when the flesh of a fish (catfish or hake) is full of tumours that are really aggregations of enormous numbers of the protozoan parasites. When this is so the flesh of the fish may resemble a kind of porridge, which runs out when the skin is cut.

Bacteria play a great part in the causation and distribution of disease among the warm-blooded animals. They are really parasites which are detrimental to the host in two main ways : (1) by feeding on the tissue substances of the body, breaking these down in very remarkable modes so that the proper functioning of the organs becomes impossible, and (2) by liberating toxins which react on the host (as in lockjaw or diphtheria, for instance). Very little has been done towards investigating the diseases of fishes that are due to bacteria and this is because of the great difficulty of obtaining thoroughly fresh specimens and, still more, of studying the course of the disease in the living fish. Three cases of undoubted piscine tuberculosis have, however, been fairly well investigated. In two of these, studied at Liverpool, the " patients " were cod affected by a lupus (a " rodent " or spreading ulcer) of the skin. The tubercle bacilli responsible for this condition produced all the effects that are seen in the typically human disease. The third case was that of a halibut investigated by Dr. Sutherland, of Bradford, and the same conditions were found here as in the cod studied at Liverpool.

But, on the whole, diseases in marine fish due to bacteria are not often observed. Ulcerated cod and plaice have been seen, in which cases the sores were, no doubt, due to bacterial infection. A peculiar condition of the skin, called " dermal catarrh " is well known in the case of fresh water fish, and this may, perhaps, be associated with bacteria. It is rather strange that fish are really very susceptible to sudden changes in the temperature of the water. This is known to be so with trout, and the fact is of practical importance when these fish are being transplanted and streams are being re-stocked. It is known also that Newfoundland cod are notably affected by sudden changes of temperature produced by the presence of ice in the waters which they inhabit.

Malignant Growths in Fishes.

Malignant growths are what are commonly called " cancers " in the human subject. Undoubted examples of these conditions have long been known in some fresh water fishes : trout, for example, are sometimes affected by a disease which is either a kind of goitre, or a sarcomatous condition. Similar conditions in marine fishes were little known until the investigations described in previous reports from this laboratory had been made.

What is really very curious is that every kind of " cancer " affecting sea fish can be exactly paralleled by a human disease. The most sinister and truly malignant of these human affections is the melanotic sarcoma, and we have now seen several very typical cases of this in fish (in ray and halibut). There is practically no difference in the microscopical appearances presented by a human and a piscine sarcoma, and one might be mistaken for the other by a pathologist who did not know from which kind of patient the microscopic preparation was made. This similarity in the ways in which the bodily tissues of such very different animals as a man and a ray can be affected is very strange, and may be of great significance in our effort to understand the nature of cancerous disease.

Malignant growths in fishes are practically always what are called sarcomas, that is, they are growths of the connective tissues. The different organs of the body are made up of different kinds of tissue : thus we have nervous tissue, muscular tissue, bone tissue, lymphatic tissue, etc. But always these organs, and their constituent tissues, are bound together, or " connected " by connective tissue which is a kind of fibrous complex—a kind of packing, or joining, or supporting substance. In the animal body it ought usually to be present in minimal quantity—never more of it than is just enough to bind together the other tissues or organs. In corpulent persons it increases largely and becomes loaded with fat and is, of course, an idle and even detrimental factor in the bodily economy.

A sarcoma is *an uncontrolled overgrowth of the connective tissue.* For some reason which we do not, in the least, know the connective tissue cells that form the packing between the other tissues start off to grow " on their own." They increase largely in bulk in some local part of the body and form a visible swelling or tumour. A visible tumour (either in a fish or in a man) is not necessarily a sarcoma ; it may be " benign " (as in the case of an ordinary " wen ") and then it is usually enclosed in a kind of sheath and so shut off from the rest of the body and restricted in its growth—*it is controlled.* But there are no bounds to the growth of a sarcoma—that is what is meant by its " malignancy." All the connective tissues of the animal body are continuous with each other, and when the sarcomatous condition appears in any part of the body it is apt to spread. The tumour has " roots " in the adjacent tissues and penetrates into the latter, and when once a typical sarcoma originates in one part it tends to appear independently even in remote parts. That is, it " generalises " or forms " metastases," or subsidiary tumours, and it is this condition that marks the gravity of a cancer ; even if removed by operation it may recur or reappear elsewhere. This power of generalisation and of forming metastases is displayed, in the most striking manner, by some fish sarcomas. In a big halibut examined at Liverpool there were one or two visible tumours, but on cutting into the flesh in other places it was seen that small subsidiary tumours were almost everywhere throughout the body. In all cases the growths were not isolated from the surrounding tissues but faded into the latter and were incessantly growing into them.

In a fish the sarcoma begins, nearly always, just beneath the skin and then grows down into the flesh. The muscle tissues never take on this uncontrolled quality of growth and it is always the connective tissue between the muscle fibres that grows. By and by the muscle substance fails to receive nutriment and begins to waste away until it disappears entirely and its place becomes occupied by the useless connective tissue cells and fibres. In the little gold-fish described in this Report this wasting, or atrophy of the muscles, had occurred all over the body and the fish had got to such a state that movement had almost become impossible—in the anatomical sense. That is, locomotion was becoming mechanically impossible because the muscles were everywhere being replaced by connective tissue.

Thus disease due to a malignant growth is in another category from those conditions that we familiarly call " disease." The condition is not due to any parasite, or bacillus, or to any condition of poisoning. The malignant growth, in its earlier stages, is essentially *healthy*. It is ordinary, typical, well-nourished connective tissue, like any other normal connective tissue. In itself it is absolutely normal, but it is simply connective tissue in the wrong place. What has happened in the animal that develops a sarcoma is a loss of control—a most typical individualistic attitude on the part of one kind of tissue. In the healthy animal (regarded as a whole) every tissue is under strict control, and there is never any more of it than is necessary in the interests of all the other organs or tissues : it is one for all and all for each, and the growth of each part of the body is regulated by the common necessities of all the other parts. But in the cancerous growth the individualism of the connective tissue becomes rampant and fatal. There is a loss of control, chemical, or nervous, or psychical, we do not know what. If we did know we should probably have the means of cure in sight.

In some cases seen—as, for example, in the sarcoma of the turbot described in the following pages—a large growth may look healthy and, on examination, prove to be clean, normal connective tissue such that one might *eat* without hesitation. But usually the stage of growth comes when the tumour becomes so big that it cannot be nourished. When a new and normal part of the animal body grows—as, for instance, in " proud flesh " that fills up a deep wound, *all* the kinds of tissue regenerate themselves in proper proportions—

there are nerves and blood-vessels, and the conditions for continued nutrition are established. But in the typical piscine sarcoma there are no blood-vessels and (probably) no nerves ; it is all connective tissue and it is ultimately incapable of healthy existence. If it is small, nutritive matter soaks into it from the surrounding tissues, but even that assistance only causes it to grow still bigger. The time comes when the tumour is so big that its internal parts cannot receive nourishment nor can they get rid of their waste products. Necrosis, or death of the individual tissue, then occurs, and the tumour begins to soften because of a process of autolysis (or self-digestion). Usually it becomes so soft that it breaks and runs into an ugly sore which cannot heal. As a rule this is the condition when a specimen is received. The difficulties of adequate investigation are enormous. One must have the living, sarcomatous fish under observation and for operation and experiment, if necessary, and the chances of this are extraordinarily small. If they could be made much greater the study of cancerous conditions would be hugely facilitated.

Diseases of Fish and Human Disease.

The question of the transmission of a piscine disease to the person who may eat an affected fish obviously suggests itself, but there can be no doubt that the chance of this happening is exceedingly small. Many grave human diseases are due to animal parasites which have entered the body in various ways. The condition called trichinosis, for instance, which is contracted by eating pork infested with the living larvae of the little worm called *Trichina.* When such measly pork is eaten the flesh is digested, but not the worm larvae, which continue to live, to grow, and to migrate from the alimentary canal into the muscles. The parasites that may give rise to human diseases are now fairly well known and it is the case that very few of them come from fish. About

52 species of Tapeworms and Flukes are now known to occur in Irish Sea fishes and shellfish, *but no one of these is known also to occur in man*, so that the risk of acquiring parasitic disease by eating fish may be neglected. In the cases of piscine tuberculosis referred to above the risk of the communication of the affection may also be neglected, for efforts were made to transmit the bacillus isolated from the cod to the tissues of guinea pigs. These experiments were quite unsuccessful. In any case, proper cooking of a fish will always kill the larvae of tapeworms or flukes contained in the flesh. It is said that round worms (Nematodes) may not be killed in the process of cooking the fish by boiling ; but if this is so, the cooking must have been a rather perfunctory operation !

The question has frequently been asked : what ought to be the procedure, on the part of the Inspectors of Markets, with regard to fish that are diseased or heavily parasitised ? It would be difficult, if not impossible, to prove that there is serious risk of the communication of disease should such affected fish be eaten and I think the position that should be taken up is this : the value of the fish, *as an article of food*, is usually greatly reduced if it is diseased and it may, possibly, even be detrimental. Ordinary parasitised fish—tapeworms or trematodes in the intestine, round worms in the intestine or body-cavity, or fish-lice on the skin or gills—in no way lower the food value or make the fish unsuitable as food. The animal may be perfectly healthy and well-nourished, although it may harbour large numbers of such parasites. But parasites visibly present *in the flesh* are another matter for, in such cases, the fish may be greatly emaciated (perhaps because of the escape of toxins into the blood-stream). Such fish flesh is not good and proper food any more than if it were perfectly healthy and preserved by very weak formalin (which renders it indigestible to some degree) and it ought to be condemned on that account, just as it would be condemned if it were stale.

It must be remembered that, in the latter case, there might be great difficulty in proving that the fish would be detrimental to the health of the person consuming it as food, yet such condemnation is the usual practice and is, of course, justified. What one buys, as an article of fish to be used as food, is something that can be eaten and assimilated and is not disagreeable—it is a foodstuff. These qualities are not exhibited by a thoroughly stale fish.

So also a fish in which there are obvious tumours ought to be condemned. The presence of such formations is certainly detrimental to the health of the fish itself, and so they lower, or very seriously impair, its food value.

JAS. JOHNSTONE.

Department of Oceanography,
University of Liverpool,
June, 1923.

CLASSES AND OTHER WORK AT PIEL.

By A. Scott.

Classes at Piel.

Only one class in Marine Biology and Navigation for fishermen could be arranged for in the spring of 1922. It was held during the period 27th March to 7th April, and was attended by the following ten men: Lawrence Bond, Charles France, John Gallimore, George Walton Haslam, Fleetwood; Thomas Cocking, Wilfred Threlfall, John Woodhouse, Joseph Woodhouse, Morecambe; Maurice Bibby, John Butler, Grange-over-Sands. Their average age was 24 years. The youngest student was 18 and the oldest 40. Mr. R. J. Daniel, B.Sc., of the Oceanography Department, University, Liverpool, had charge of the whole of the teaching work as in previous years.

Attempts were made to fix up other classes for Manchester University Senior Students, members of the Manchester Microscopical Society, and a summer course for school teachers, but the response, mainly because of the high cost of travelling, etc., was insufficient to enable us to complete the arrangements. There appears to be no doubt that similar classes which were carried on in the previous year had created a good impression. Quite a number of these former students who happened to be holidaying in the Lake District revisited Piel at intervals during the summer and all spoke of the useful work thay had done in the class.

Student Workers.

Mr. Owen Hunt, one of the senior students in the Zoology Department, University, Manchester, who worked at Piel in 1921, revisited the establishment for a week in April, 1922, just after the fishermen's class came to an end. He was mainly engaged examining the samples of preserved plankton and also living material collected by tow-net in the channel. Later on he graduated B.Sc., and was appointed to the staff of the Marine Biological Association's Marine Laboratory at Plymouth. B. Sundara Raj, M.A., Assistant Inspector of Fisheries, Madras, who is taking a two years' course in the Oceanography Department, University, Liverpool, under Professor Johnstone, spent two weeks at Piel when the fishermen's class was being carried on. He was thus able to see the system that has been developed by us for instructing the men in Marine Biology and Navigation. He also carried out research work. Mr. Raj revisited Piel at the end of the year to study the methods of examination and identification of plankton organisms and also the general drift of shore deposits.

Visitors.

The Mayor, Town Clerk, and members of the Bury Corporation, inspected the fishermen's class on 31st March. On Saturday afternoons throughout the summer months many visitors came to see the living invertebrata, etc., in the tanks and the preserved specimens used for teaching purposes. The visitors included school teachers from Barrow, accompanied by their classes, the members of the Barrow Naturalists' Field Club, the members of the Barrow Technical Students' Union, etc. When the party was a pre-arranged one a lantern demonstration was usually added to the programme.

Fish Hatching.

A temporary failure of the gas supply at the end of March, just as the plaice were beginning to spawn, forced us to abandon hatching work in the spring of 1922. The large plaice were at once turned into the channel. Spawning had begun on 23rd March, and the few thousands of eggs obtained between that date and the end of the month were hatched out in the apparatus and the larvae liberated in due course.

The Barrow Corporation eventually decided on an electricity supply for Rampside and Roa Island and also to carry on the supply of gas until the electricity scheme was completed. The gas supply was only a temporary one, as the little local installation so long worked by the Railway Company mainly for lighting the channel was too old to warrant any expense on its renewal. It might give out at any time, and it was unsafe to begin experiments requiring a constant supply of sea water. The small wood tanks and glass aquaria were re-stocked with various local invertebrata such as anemones, star-fishes, sea urchins, crabs and zoophytes. These invertebrates enabled us to show visitors something of the life on the seashore between tide-marks. There was considerable delay with the electricity supply and the scheme was not completed until February, 1923. The gas plant gave out at the end of the year and the contents of the tanks had to be turned into the channel. Owing to the uncertainty of motive power to work the pumps no spawning fish had been secured, so that the loss was not so vital as in the spring of 1922. The establishment has now been fitted up with electric light. A 2½-horse-power Westinghouse D.C. motor with a B.T. self-starter has been installed to drive the pumps in place of the Crossley gas engine which had been in use for twenty-six years. The motor is proving very satisfactory. The pumps fill the store tanks with 5,000 gallons of sea water in two hours at a very small cost for current.

SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF THE MUSSEL (*MYTILUS EDULIS*).

(*Concluded*).

By R. J. Daniel, B.Sc.

(With 6 Plates).

In two former papers upon the chemical composition of the Common Mussel, based on estimations of fat, carbohydrates and proteid, made monthly for over a year*, references were made to microscope slides prepared with the object of showing the distribution of fat and glycogen in the tissue. These sections represent a complete series throughout the year, and it is proposed now to discuss them more fully.

Since the enquiry leads into the examination of cellular structures, it is well to review briefly the histology of the tissues which are most nearly concerned.

The Alimentary Canal and Digestive Gland.

The alimentary canal, as seen in Pl. I, fig. 1, consists of a stomach, embedded in the digestive gland, from the posterior end of which the direct intestine runs back to the posterior adductor muscle. Here it gives rise to a recurrent intestine, which after forming a loop in the digestive gland, turns back again, and after passing through the dorsally-situated heart, has its anal opening on the dorso-posterior side of the posterior adductor.

The direct intestine communicates by means of a long narrow slit with the caecum, which contains the crystalline style. The alimentary canal, for its whole length, is lined with ciliated columnar epithelium, and the absorbing surface of this is increased by various convolutions, which are particularly marked in the stomach region. The digestive gland, or so-called "liver," envelops the stomach and occupies the anterior part of the visceral mass. It exhibits a varying number of main canals (about 12) opening into the stomach. The lateral branches arising from these give off numbers of blind sacs, which ramify throughout the surrounding connective tissue.

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* Daniel. "Seasonal Changes in the Chemical Composition of the Mussel," *Lancashire Sea Fisheries Laboratory Reports*, 1920, p. 74; 1921, p. 205.

The main ducts are lined with epithelium, ciliated in part, and are bound by circular muscle fibres.

The columnar epithelium of the secondary canals and blind tubules is not ciliated. The cells are granular in appearance, with large nuclei, and provide a cuticular lining to the lumen of the canal (Pl. IV, figs. 1 and 2). It is in these cells that the green or brown granules, which give the characteristic colour to the digestive gland, are found, and are apparently derived from food material.*

Often, smaller, more rounded cells, are wedged between the bases of the granular cells, and they never abut on the lumen. Once thought to be "ferment" cells, they have also been described as younger granular cells,† and seem to take an equal share in the storing of fat.

The Connective Tissue.

The connective tissue in section has the appearance of a network with uneven meshes, the strands of which vary in thickness (Pl. II, fig. 1) from slender threads to a solid hyaline substance which, in sections, may cover a larger area than the "spaces" they enclose as in certain parts of the mantle edge. Pekelhering‡ has shown in the oyster that this substance may be differentiated from the thin wall which surrounds these "spaces." Langer thought these latter were actually empty and called them vesicles. Although this is now known to be incorrect, they have been called Langer's vesicles by later writers, and therefore will be referred to as such in this paper.* Flemming was first to recognise in them a cellular structure, and in spite of a certain amount of controversy this view is now generally accepted.† The nucleus of the Langer's vesicle is easily demonstrated by fixation in corrosive sublimate and acetic acid, or picro nitric acid with after staining in methyl. blue eosin, or haemalum eosin. The nucleus is eccentric in position, sometimes lying close to the cell wall. An intercellular network of fine threads radiates from the position of the nucleus towards the periphery of the cell. It is difficult to demonstrate whether or not these strands are directly connected with the nucleus.

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* C. A. MacMunn. *Phil. Trans.*, Royal Society, London, Vol. 193, 1900.

† C. A. MacMunn. *ibid.*

‡ Pekelhering. "Onderzoeking gedaan in het Physiol. Laborat. der Utrechtsche." *Hoogeschool* (5), III, 1. 1901.

Typically, the connective tissue in the mussel does not, however, consist of these Langer's vesicles, surrounded by a greater or less amount of clear inter-cellular substance. Lying between the Langer's vesicles, and packed in such a way that they often appear to define the boundaries of these bodies, are highly granular cells, each with a small definite nucleus. They may be so massed together that the cell individuality is apparently lost, and they appear as a granular mass containing several nuclei (Pl. II, fig. 1). These so-called "round cells" of Kollman are often extremely angular in contour and may assume a comparatively large size.

It is the Langer's vesicles and round cells which arrest the attention in the study of reserve products, and other cell structures of the connective tissue need not be touched upon, since they have played no part in the present enquiry.

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* It may be noted, however, that Kollman objects to this nomenclature, and says that cells which have been called Langer's vesicles really compare with the spherical cells of other molluscs. M. Kollman, "Recherches sur les Leucocytes," etc. *Annales des Sciences Nat. Zoologie,* Ser. 9, VIII, 1908, p. 67.

† For summary see T. List, "Die Mytiliden." *Fauna und Flora des Golfes von Neapel,* XXVII, 1902, p. 107.

The Reproductive Organs.

It has been shown that the chemical changes which take place in *Mytilus edulis* during an annual cycle are intimately bound up with the activity of the reproductive organs. The sexes are separate, but in male and female there is an invasion by the reproductive elements of other tissue in the body. The permanent portion of the reproductive organs is bilaterally symmetrical, and has an external opening, the genital papilla, situated on each side of the body, a little in front of the posterior adductor muscle, between the mesosoma and the inner gill base (Pl. I, fig. 1). From each genital opening, a duct runs forward to below the pericardial cavity, and then gives off numerous lateral branches which end in follicles in all parts of the body and mantle. The main duct is lined with ciliated epithelium, which is partly replaced by germinal epithelium in the tubules. The male follicles, in cross section (Pl. V and VI) measure up to 0·5 mms. The female follicles are larger and fewer in number.

In a mussel about to spawn, the tubules and follicles ramify practically throughout the whole body of the animal, impinging upon other organs, and growing at the expense of the connective tissue. It is this interplay between the reproductive products and the connective tissue which decides the amount and the distribution of the fat and glycogen present in the animal. Before passing to a description of this distribution as shown in various sections, however, it is advisable to describe the different methods employed in obtaining the sections themselves.

Material and Method.

Certain mussels were laid aside from each monthly sample and removed alive from their shells. Slices of tissue, 3-4 mm. thick, were cut in transverse section by means of a "safety" razor blade, from a position in the body, immediately anterior to the "foot," between A and B, Pl. I, fig. 1. These were placed in the various fixatives referred to below.

The microscope sections finally obtained from the different samples, therefore, may be compared with ease, since they have been made through the same organs and tissues. They are appropriate to the enquiry, because they all pass through parts of the digestive gland and connective tissue of the body and mantle, and contain the cells which are most actively concerned with the storing of reserve products.

Fixation and Staining for Fat.

The tissue was fixed in 5-7 per cent. formaldehyde and subsequently embedded in gum mucilage or 33 per cent. gelatine in water. Transverse sections about 0·01 mm. thick were cut by a freezing microtome. They were stained in a saturated solution of Sudan III in 70 per cent. alcohol, differentiated for five minutes in 70 per cent. alcohol, and mounted in glycerine jelly.

These sections figured in Plates V and VI give an excellent general view of fat in the "liver" and connective tissue. Pieces 1-2 mm. thick were also fixed in the following mixture:

*10 % aqueous solution of potassium bichromate	100 ccs.
Glacial acetic acid	5 ccs.

at 30°-40° C. for 24-48 hours, washed in running water for twelve hours, embedded in paraffin, and after staining in alcoholic Sudan III for ten minutes, mounted in glycerine jelly or Canada balsam. Tellyesniczky's fluid (a weaker bichromate mixture) is also effective, and seems to be superior as a histological fixative.

The sections give practically the same picture as the frozen ones, although at times the method seems somewhat capricious. One is able to obtain, however, a thinner section

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* Bell. *The Journal of Path. and Bacteriology*, Vol. XII, No. 1, 1914, p. 105.

(4-6μ) and see more clearly the distribution of the fat in individual cells.

Fixation and Staining for Glycogen.

The tissue was fixed in several changes of absolute alcohol and mounted in paraffin. The sections 6μ thick were stained in Lugol's solution of iodine and potassium iodide, dehydrated in absolute alcohol saturated with iodine, and differentiated in oil of origanum. This is the general procedure adopted for the histo-chemical identification of glycogen, and is useful in two ways. The sections obtained serve as a comparison with other fixatives and stains used. Further, it is easy to prepare check sections by passing one set of slides through water containing saliva. This hydrolyses the glycogen, which dissolves out before staining with iodine.

Results obtained in this way may compare favourably with other methods used, but alcohol is not a good fixative, especially for such structures as the connective tissue of the mussel; moreover, the iodine stain fades rapidly in this mollusc. This may be prevented to some extent by mounting in vaseline, or removing the oil of origanum with xylol before mounting in Canada balsam.

If necessary, the sections may be restained by "sweating" off the cover slip with xylol, and immersing them again in Lugol's solution.

Several other methods for demonstrating glycogen were also tried. Probably these were originally devised for mammalian tissue, and in the present enquiry met with no great success. Feissinger *advocates the use of an aqueous solution of tannin, followed by potassium bichromate. This treatment fixes glycogen in the mussel, but suffers from a lack of penetrating power. In small and thin pieces of tissue, the zone of fixation is only two or three cells deep, and even these have

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* N. Feissinger. *C.R. Soc. Biol.*, 1909, p. 183.

not a normal appearance. Mayer[*] has written upon various stains for glycogen and recommends the use of Vastarini Cresi's fuchsin and resorcin,[†] and also gallic acid ink.[‡] In my hands, with *Mytilus*, these give only a diffuse tissue staining, and the manipulation required for the former stain is a further disadvantage. Best's carmine, however, was found to be an excellent stain, and its specificity is comparable to that of iodine. The mixture deteriorates rapidly, but the trouble involved is well repaid. This was the only stain consistently used, other than iodine.

It is obviously an advantage to have both fat and glycogen stained in the same section. To this end Buchner[§] advocates the use of equal volumes of Flemming's chromo-osmic fixative and absolute alcohol, to be mixed immediately before use. With mussel tissue this mixture has proved very successful. The blackening of the fat with the osmic acid gives results comparable with the sections fixed in dilute formaldehyde, and stained with Sudan III, and the glycogen stains equally well after this fixation, with iodine[||] or Best's carmine. The absolute alcohol acts as a diluent to the more expensive fixative, and from this point of view it is valuable in routine work, when the liquids may be used in quantity. Its presence, however, is not necessary for the fixation of the reserve substances. Flemming's chromo-osmic mixture (and indeed osmic acid alone) fixes both fat and glycogen, used either in its original form, or as modified for cytological work (without acetic acid).

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[*] P. Mayer. *Zeit fur Mik.*, 1909, XXVI, p. 4.

[†] Vastarini Cresi, "Recherches ultérieures sur une nouvelle méthode de coloration du glycogéne dans les tissus." *Atti della R. Med. Chir. di Napoli*, 1909.

[‡] Silbermann and Ozorovitz. *Bul. Soc. de Stinte Bucarest*, XVII, 1908.

[§] Buchner. See reference, *The Microtomist's Vade Mecum.* Lee, 8th edition, p. 296.

[||] P. Mayer. *ibid.* Has pointed out that strong alcoholic solutions of iodine demonstrate glycogen as a homogeneous substance, and that this is probably due to solution of the glycogen. I think this is less evident after Flemming.

Bell's potassium bichromate mixture and Tellyesniczky's fluid fix glycogen to some extent, as well as fat, and this suggests that a suitable combination of stains might be used to demonstrate both substances in the one slide.

The Distribution of Fat in Sections.

For the quantitative estimation of the "fats," carbon tetrachloride was used in a Soxhlet apparatus, and the estimated results are referred to as "carbon tetrachloride extract."* This certainly contains several fatty substances.

A further general separation of fats from the tissue was obtained in the following manner:—Dried tissue was taken and extracted in cold and hot alcohol, with the aid of constant shaking. After filtering, the liquid was evaporated off on the water bath. The residue was then dissolved in ether, and this was allowed to evaporate until a syrup was formed. The addition of acetone separated out the insoluble phosphatides, which represent about one-half of the "fat" present. For instance, in April, at the time of spawning, 58·6 per cent. of the alcohol extract may be insoluble in acetone.

The question naturally arises as to how far these two substances react to the fixatives and stains used for demonstrating fat in the sections.

Sudan III and Flemming's chromo-osmic mixture were generally used, and both demonstrate almost exclusively the true fats. The latter are the only fatty substances they stain in common, and since the distribution of globules in both series of sections are comparable, it may be assumed that whenever fats are mentioned in the following pages the reference is to true fats.

Lecithin also is supposed to stain with Sudan III, though less intensely.† In examining sections of mussel, the differences in colour of the various fat globules after staining is very striking; they may grade from deep red to pale yellow. This is no proof, however, that the phosphatides are stained, and indeed, if sections are passed through acetone before using Sudan III the results are negative.

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* Daniel. *Lancashire Sea Fisheries Laboratory Report*, 1921, Tables III and IV, pages 219, 220.

† See, for instance, *Technique Microscopique* (Böhm and Oppel). Rouville, 5th edition, p. 366.

After fixation with potassium bichromate, the fat, stained with Sudan III, appears as solid globules. Bell* claims that in the human kidney solid spheres thus stained are lipoids. This is apparently not the case in *Mytilus* because the appearance and distribution of fat is much the same as in the formalin sections. If these latter sections are treated with Nile blue sulphate instead of Sudan III, similar fat globules are stained; they are blue in colour, however, showing an absence of neutral fat. In addition the sperm sac contents are stained a deep blue. Both Nile blue sulphate and neutral red are stated to stain phosphatides† and certainly after acetone both colour the sperm sacs deeply.

The testicular follicles also blacken after osmic acid, and this colour may be easily removed with turpentine. This reaction strengthens the possibility that the sperm sacs are one of the sources of the phosphatides. It cannot be the sole one, however, since these fatty substances may be extracted in quantity from tissue in September, when the reproductive products are negligible in mass.

The staining of phosphatides in other parts of the body presents some difficulty,‡ which might be explained if they are closely bound up with the other types of fat; nor has it been possible to obtain positive results with polarised light.*

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* E. T. Bell. *ibid.*

† Aschoff, "Zur Morph. der Lipoiden Substanzen." *Beit. zur Path. Anat.*, XLVII, 1910, p. 1.

‡ For methods of staining different fatty substances in tissue the reader is referred to:—

Ziveri, "Über die Natur der Lipoiden Abbaustoffe des Zentral nerven Systems in ein Pathol. zuständen." *Fol. Neuro Biologica*, Bd. VI, 1912, No. 9, p. 719.

M. G. Loisel, "Essai sur la Technique comparative de la lécithine et des graisses neutres." *C.R. Soc. Biol.*, Vol. 55, 1903, p. 703.

Fat in the Sections.

Alimentary canal: the parts of the Alimentary canal shown in section (Plates V and VI) include the stomach. direct intestine, and the recurrent and terminal canals. All these show quantities of fat globules after staining with Sudan III or Flemming's fixative. They lie in the cells of the lining epithelium, or when crowded together, may take up a position between them. If the epithelial cells are comparatively long, as in parts of the direct intestine, globules may be present throughout their whole length (Plate I, fig. 3).

The fat may be concentrated in such a manner that, after a staining reaction, it represents bands of colour, more or less broken, lying in the epithelium. These bands often occupy the basal, or central portions of the cells, but may also appear immediately below the cilia, in the distal parts abutting the digestive canals (Plates V and VI). They suggest vigorous periodic feeding, with alternate "rest" periods. The large amount of fat present, however, is against this idea, and it may well be that the epithelium of the alimentary canal acts as a store for this reserve product.†

The figures in Plates V and VI represent corresponding parts of sections cut with the freezing microtome, and stained with Sudan III. They aim at giving a "bird's eye view" of the distribution of fat in various tissues, during different months of the year. The drawings are not made to a high magnification, and it has been necessary, therefore, to exaggerate the size of the fat droplets, and at the same time to reduce their numbers. This work has been done with some care.

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* Aschoff. *ibid.*

† Mussels kept in tanks and "starved" still show fat in the canal epithelium.

however, in order to give as correct an impression as possible as to the general distribution of the fat. In order to give a more precise idea, as to the actual arrangement of the fat, tissue has been drawn, in the right-hand corner of each figure, to a higher magnification, and in accurate proportion. It represents either an enlargement of a part of the section it accompanies, or has been obtained from a corresponding section, for the same month, after Bell's method.* The advantage of the latter is that a thinner section may be obtained, and the contents of the individual cells may be seen more clearly, although the "round" cells of the connective tissue still appear as a reticular network.

These figures show that when suitable methods are employed, fat may be demonstrated in the epithelial cells of the alimentary canal at any time of the year. It is interesting to note, in connection with this, that the guts of all mussels used in the histological work contained a quantity of ingested material, irrespective of the time of year. Signs of constant feeding, however, are not always general. Herdman and Scott† in a routine examination of the gut contents of mussels from the Morecambe Bay area, made some years ago, found them to be empty in samples from May 30th to August 7th. There are other samples scattered throughout the year, which give the same negative result.

It will be seen from Plates V and VI that there is an appreciable amount of fat in the epithelium of the gut during September and October. In December the amount shows a marked increase, in conjunction with a rapid development of the reproductive products. There seems to be an increased amount of absorption from January until the time of spawning in April, and this does not decrease again until August.

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* Bell. *ibid.*

† Herdman and Scott. *Lancashire Sea Fisheries Laboratory Report*, 1894, p. 40.

The Digestive Gland, which, according to List,* functions primarily as a storehouse of reserve food material, also shows a supply of fat throughout the year. The latter is present in varying quantities in the acini along with pigment from the food ; this connection is clearly seen in potassium bichromate sections where the Sudan III stain is not so diffuse as in the frozen tissue. The amount of fat in the acini varies very much and it is not unusual to find sections of neighbouring tubules, which differ markedly in their content : some exhibiting the characteristic green pigment, and little or no staining reaction, while others may be of an uniform deep red when treated with the Sudan III.

In the figures, the digestive gland is drawn in outline, and only those tubules are drawn which show a decided staining reaction. When the fat globules are present in quantity, a tubule is represented by a thick continuous outline, but if it contains a lesser amount it is represented by dots. The primary tubules ramify through the tissue and may present longitudinal or sagittal sections which are represented in the drawings as pairs of lines. They show a vivid staining, due to the presence of large globules, for it is here, rather than in the blind sacs, that the fat is found. The relative amount of fat in the digestive gland is greater than in any other tissue, and it does not seem to vary with the reproductive periods. One might expect the store to be drawn upon very heavily during the growth of the sexual products, but this, apparently, is not the case. For instance, sections for January and February show the gland well stocked and comparable with the conditions of the tissue in June and August, when the animal is building itself up again prior to the next spawning season. The fact that there is probably more food on the beds in spring (the time of the diatom maximum) may explain in part why the demands made by the gonads at this time upon material and energy do not impoverish the reserves of fat in the digestive system.

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* List. *ibid.*

The Connective Tissue.

Fat droplets may be demonstrated in the Langer's vesicles by means of Flemming or Champy fixatives (Pl. I, fig. 5). They generally lie near the periphery of the cell, and may be seen apparently clinging to the hyaline substance between the cells, when no round cells are near. This tendency to close upon the outer cell wall (shown also by the nucleus) is so marked in some cases that when round cells are present it is often difficult in section (5-6μ) to know whether the droplets belong to one type of cell or the other. It is the round cells, however, which are mainly concerned with the storing of fat. At all times of the year, their granular contents contain it, and at times the globules are so numerous that they may coalesce (Pl. I, fig. 5). In May, after spawning, when residual ova or sperms still remain in the thin and wasted animal, there is little evidence of fat in the round cells, except in the region of the remaining sexual products. The connective tissue, however, recovers rapidly from the disorganisation brought about by the invasion of the reproductive follicles. In June* the mantle has thickened considerably, and there is evidence of an increase in the round cells and their fat content, which continues during August and September. The amount of fat is augmented in these two months, by the appearance of accumulations which lie in the tissue and stain very deeply with Sudan III (Plates V and VI). They are more solid in appearance than the grouping of fat one finds round the veins and artery walls, and seem to mark the beginning of the growth of the new follicles of the gonads. At least such is suggested by the appearance of the section in September when the reproductive tubules have developed enough to be traced under the high power. The round cells are now well packed with fat, and this may help to explain the increase in general fat contents shown by Tables III and IV.* The ovarian follicles appear in October, closely packed with young ova which show no reaction to staining for fat. They are almost fully grown before the globules appear. From November onwards the products of the gonads multiply rapidly in size and numbers. The round cells become jammed between the follicles, and there is little doubt that the fat storage in the connective tissue is being augmented to meet this extraordinary activity of the reproductive organs, and supply reserve material for their products. Considering the small percentage of fat contained in mussel tissue, the increase immediately before spawning is very striking.†

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* This refers to 1921, during which year the sections figured in the Plates were prepared. In 1920 the exhaustion would seem to have lasted for two months longer. See Daniel, *Lancashire Sea Fisheries Laboratory Report*, 1921, Table III, p. 219.

Glycogen in the Sections.

Microchemical staining shows that glycogen exists in small quantities in practically all tissues, except those of the gastric gland, and the epithelium lining the alimentary canal. Its concentration in the Langer's vesicles of the connective tissue is very marked, and the demonstration of its presence is simple. Gross sections of tissue removed fresh from the animal, or after fixation with absolute alcohol, may be placed in a watch glass and covered with Lugol's solution. The staining process may now be watched under the low power of the microscope. As the liquid penetrates the cells, it imparts its own yellow brown colour to the latter, but the deep mahogany colour which is characteristic of the reaction between iodine and glycogen manifests itself in Langer's vesicles. They appear now as a number of brown isolated bodies, suspended in a

* Daniel. *ibid.*

† Daniel. *ibid.*, Table III.

yellow network. The amount of glycogen which occupies neighbouring vesicles, in section, varies; it may almost completely fill the cell, or apparently be absent altogether. These conditions are shown in Plates III and IV. It is somewhat difficult to ascertain in what form the glycogen exists in the tissue cells, before fixation. The examination of fresh material under the microscope is hampered by the granular nature of the round cells.

If fresh tissue is pressed gently, when sexual products are absent, a good deal of liquid runs from pieces of the mantle, and the quantity is greater than could be supplied by the blood vessels. This liquid is opalescent, very much like the blood plasma in appearance, and suggests that the glycogen is in solution. Fixation alters the appearance of the cell, but comparison of Langer's vesicles after various mixtures, gives some idea of the original contents.

A routine fixative, such as chromo-nitric acid, does not preserve all the cell inclusions. The nucleus of the vesicle is present, but apart from this, the only other structure visible, as pointed out above, is a fine network of strands (Pl. II, fig. 1). It is in this way that the cells have generally been figured. Fixation by dilute formaldehyde gives the cell contents the same "stringy" appearance in thin sections (3μ), but if thicker ones are taken (6-8μ) the groundwork tends to show up as a homogeneous mass. Strong Flemming (modified) gives a stout reticulated network, easily made out before staining, which may have a very different appearance in adjacent cells. The network may be very fine in some, while, on the other hand, the contents may appear contorted and broken up into separate parts, suggesting a violent precipitation by the fixative (Pl. I, fig. 5).

Fixation by 2 per cent. osmic acid for seven days gives a more closely spun reticulum, which again shows variation and may represent a granular structure. The appearance of the vesicles after these fixatives suggests a fluid or semi-fluid solution which becomes coagulated, or from which a precipitate is thrown down. Nor is the substance that is laid down necessarily all glycogen. Staining with Lugol's solution shows this. The network is yellow after Flemming's fixative and may be plainly seen under the microscope. When stained with the iodine solution, the glycogen is often found to have a restricted distribution (Pl. I, fig. 5). In cells with contorted contents, however, staining may be general.

The storing of glycogen is an important rôle of the Langer's vesicle. The carbohydrate may be always demonstrated in fair quantity in any mussel, whatever its condition. Wherever in the animal, connective tissue exists, the vesicles will be found to contain it, as for instance in the labial palps (Pl. I, fig. 2). Yet the amount of carbohydrate varies throughout the year. This has been shown in Table III* and the reason given was the gradual encroachment of the reproductive products, at the expense of the connective tissue of the body and mantle. The figures on Plates III and IV give strong support to this view, but they only represent the two extreme conditions, in a series of microscope slides which have been prepared throughout a year, and represent how completely the connective tissue cell and its contents are subservient to the needs of the gonads.

The appearance of the mantle in the month of August, when the amount of carbohydrates in samples was found to be at a maximum, may be seen in Pl. III, fig. 1. The Langer's vesicles take up a considerable part of the connective tissue, may measure in diameter 0·1 mm. and the sections rarely show one of these cells in which the presence of glycogen cannot be detected. With Best's carmine or Lugol's solution, the majority show the glycogen, in large irregular masses, often impinging upon the cell wall, or else scattered within the cell as rounded droplets of various sizes which, before staining, have the smooth appearance of fat globules.

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* Daniel. *ibid.*, Table III.

Except for the presence of the ramifying gastric gland tubules, the connective tissue of the body is the same as that of the mantle. In fact, this is true of all the sections cut. The invasion by the gonads is the same in body and mantle throughout the reproductive cycle.

The marked change that has come over the connective tissue, by the time February is reached (two months before spawning), is shown in Plates III and IV, fig. 2. The tissue is restricted by the enormous growth of the sperm sacs. The vesicles are not only smaller and more crushed together, but there is actually less glycogen in them. In parts, the connective tissue may show a complete breakdown. A further step in this degeneration is seen in Pl. II, fig. 2, which shows a typical part of the section across the body of a female mussel, in April, at the time of spawning. There is an absence of tissue between the eggs in the follicles, and it is difficult to pick out the remaining connective tissue—which reveals itself as little isolated patches, showing few Langer's vesicles, and these with little glycogen content. In spite of this, the percentage of glycogen shows an increase immediately before spawning, which may be due to its appearance in the mature reproductive products. This has not been *clearly* demonstrated in section, even with Kan Kato's* iodine method, and is not therefore figured in the drawings, but nevertheless glycogen may be separated from tissue in considerable quantities, when no histo-chemical staining reactions are obtained.

Examination of the sections prepared during the intervening months, between August and the time of spawning, shows the gradual diminution in the number of the vesicles, and micrometer measurements on the whole also a decrease in size. The glycogen, however, manifests itself in much the same forms throughout the year.

* Kan Kato. *Pflüger's Arch. Ges. Physiol.*, 1909, pp. 125-142.

It is during October that the reproductive organs first show themselves definitely in the connective tissue, and from this time onwards until about a month before spawning, the percentages of the carbohydrates show a steady decrease. As a contrast, and allowing for fluctuations, the percentage of the proteid increases throughout the year until the time of spawning, and the same may be said of the actual weights. This is true also of the fats, so that the proteid and fat content of the mussel is rising, when the carbohydrates show a decided decrease. This is the indirect evidence that glycogen assists in the building up of the reproductive bodies, and their accumulated fat, and it seems to be supported by the examination of the tissue in section. The change of carbohydrates to fat is known to take place with ease, and Matthews* suggests that this reduction may assist in the respiration of the cells concerned. Certainly in the case of the thickened mantle of the mussel, such an arrangement would seem to be desirable.

Rôle of the Connective Tissue.

The impression one obtains, then, from sections treated to show fat, or glycogen, or both products, is that the connective tissue has taken upon itself the function of storing reserve food material. In this work, the two main types of tissue cell, the Langer's vesicles and round cells, take a prominent part. The vesicles are instrumental in storing carbohydrate material as glycogen, the granular round cells are the storers of fat. They do not carry on these functions to the exclusion of each other, for fat globules are found in Langer's vesicles, and there may well be small amounts of glycogen in the round cells; but the main purpose of the two cells is clearly defined. Both types of cell have to yield to the rapid growth of the sexual follicles. They are crushed and eventually almost obliterated by the latter, and it is natural therefore to assume that such reserve material as they possess is used up by these rapidly growing elements.

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* A. P. Matthews. *Physiological Chemistry,* 3rd edition, 1921.

Sections fixed in Flemming or stained with Sudan III show the ova lying in their follicles, and often abutting on the round cells. The fat globules look as if they might have been transferred directly from one to the other. Such, of course, is not the case; there are differences in the " make up " of the fat. If, for instance, sections fixed in any chromo-osmic mixture are immersed in turpentine, the osmicated fat granules of the ova are dissolved out in ten minutes, whereas it is a question of hours (or days) before this liquid has any effect on the fat of the round cells, or the digestive gland. A difference which is much more striking, however, is the abundance of glycogen in the Langer's vesicles, and the apparent lack of it in the sperms and ova. If present in the latter bodies it does not exist in the same abundance.

The Blood.

The amount of blood present in a mussel is relatively great.* When tapped from the body it shows itself as a liquid of marked opalescence, and this gives the typical " port wine " reaction to iodine solution which is associated with glycogen. If centrifuged so that the leucocytes are separated from the plasma, the latter retains its opalescence, and glycogen may be thrown down from it, by the addition of an excess of alcohol. If the leucocytes are fixed in osmic acid vapour, they blacken and suggest the presence of fat. The fact that they stain easily with iodine solution† suggests the presence of glycogen, either stored within themselves, or absorbed from the plasma.

The blood is brought into close contact with the connective tissue, through ramifications of arteries and thin walled veins, and may therefore be a source from which the reserve materials of the tissue are obtained. This relationship has been suggested before, in two very different ways. Sabatier* states that the cells of his "adenoid" tissue, which lie at the base of the stomach epithelium, and from which they obtain their fat, pass into the blood stream and form the "globules" of blood. Kollman,† on the other hand, maintains that the round cells of the connective tissue are themselves derived from the blood leucocytes.

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* I. A. Field (*Bul. Bureau of Fisheries*, Vol. XXXVIII, 1921-22, p. 155) estimates that a well-nourished mussel 3 in. long contains 5-6 cc. of blood.

† E. S. Goodrich, " The Pseudopodia of the Leucocytes of Invertebrates." *Q. J. Mic. Science*, LXIV, 1920, p. 19.

Note on other Cell Inclusions of the Connective Tissue Cells.

While engaged in the examination of material fixed in modified Flemming, small half-moon shaped bodies were observed, generally existing in pairs, in the Langer's vesicles. They were observed upon occasion also in the round cells, but it cannot be definitely stated whether they were in these cells, or belonging to vesicles below them in section. The bodies were yellow or orange coloured after the fixative and took no part in the subsequent staining for glycogen (Pl. I, fig. 5). They were not found in material fixed by Mann-Kopsch or Champy-Kull methods, but appeared after fixation in neutral formalin, and staining with iron haematoxylin. Their shape suggests them to be Golgi elements, and since they occur in cells which contain glycogen, it is interesting to refer to a paper by Kolatchev‡ upon the "Cytology of the Nerve Cells of Molluscs." This writer states that in these cells, glycogen appears first in the concave portion of Golgi elements, and when it increases in amount, no Golgi apparatus is apparent.

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* A. Sabatier. *Annales des Sciences Naturelles*, 1877, Series 6v, p. 22, P. 7, fig. 1.

† Kollman. *ibid.*, p. 67.

‡ A. J. Kolatchev, "Recherches Cytologiques sur les cellules nerveuses des Mollusques." *Archives Russes d'Anatomie d'Histologie et d'Embryologie*, Vol. I, 1916-17, p. 383.

He concludes that the apparatus plays a part in the formation of glycogen. So far as the mussel is concerned, I have had no opportunity of pursuing this question further.

The sections fixed in neutral formalin also show a number of small rounded bodies in the vesicles, which stain deeply with iron haemotoxylin, and suggest mitochondria. There is, however, little supporting evidence in favour of this. Certain bright red bodies appear after Altmann's acid fuchsin and picric acid, but the other contents of the Langer's vesicle, including the glycogen, stain so deeply by this method that it is difficult to form any conclusion about them.

EXPLANATION OF PLATES.

PLATE I.

Fig. 1. *Mytilus edulis* dissected from the left side after removal from the shell. The position of the gastric or digestive gland is shown, and also the alimentary canal. The terminal intestine runs through the pericardium and the ventricle of the heart. The sections shown in Plates V and VI were obtained from the region lying between the two lines A. and B.

Fig. 2. Longitudinal section of a labial palp. The connective tissue consists mainly of Langer's vesicles containing glycogen. The round cells typical of connective tissue in the mantle and body are not in evidence. The ridges of the palps contain granular bodies apparently cellular in structure.

Fig. 3. The lumen of the direct intestine may be almost divided into two separate canals, by outgrowths of elongated epithelium, such as the one depicted in the figure. The larger canal contains the crystalline style. The fat globules are shown scattered about

in the epithelial cells, and may be transferred to other tissues of the body by amoebocytes, which List has demonstrated in the Mytilidae.

Fig. 4. Suitable histochemical methods show the presence of glycogen in the gill filaments. Some of the glycogen shown in the illustration may be from the blood in the branchial vein, since the blood fluid contains this substance. There is evidence, however, that it is natural also to the gill tissue itself.

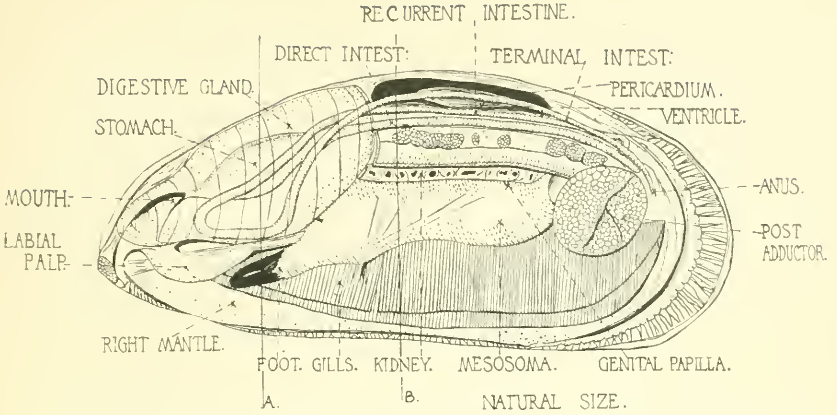
Fig. 5. Typical cells of the connective tissue as seen in the body and mantle, under an oil immersion lens. The granular reticulum is composed of " round " cells which have coalesced, and contain a number of fat globules.

In this and subsequent figures, the fat is shown as black droplets or masses, and glycogen is always represented by cross hatching. It will be seen, therefore, that in this section, glycogen is restricted to the larger type of cell, or Langer's vesicle. Its distribution is not uniform in the various vesicles. It may be restricted to one part of the cell, as in the centre of the figure, or spread throughout as an irregular network. There is a disposition for the contents of the Langer's vesicle to become broken and scattered in appearance after fixation. In a well-preserved cell, small half moon shaped bodies may be seen existing in pairs, and suggesting in appearance Golgi elements. Small rounded bodies apparently refractive under transmitted light may also be present. It is difficult to say what these bodies are ; they seem to give no reaction to staining nor show under polarised light.

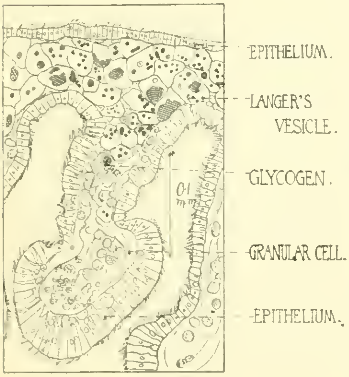
Plate II.

Fig. 1. A thin section of connective tissue of the mantle. Fat and glycogen have not been preserved in fixation, and the positions of the fat globules in the round cells are marked by vacuoles. The contents of the Langer's vesicles appear as thin broken networks of protoplasm.

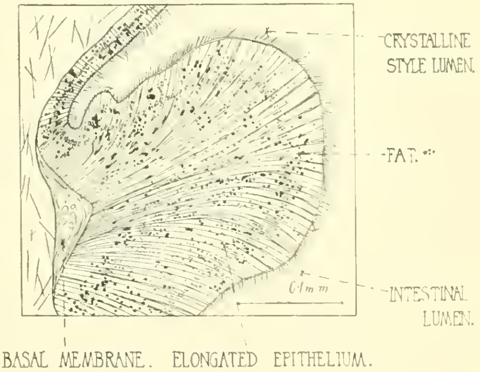
PLATE I



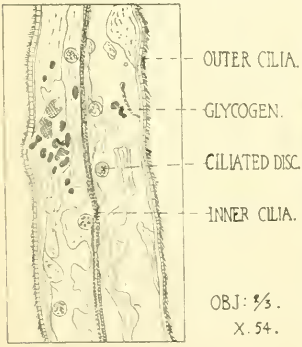
1. MYTILUS EDULIS: LEFT MANTLE AND GILLS REMOVED. ALIMENTARY CANAL DISSECTED OUT.



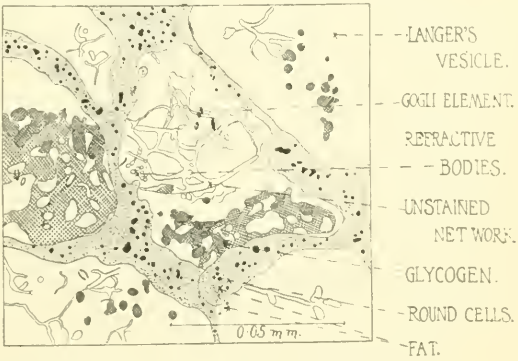
2. LONG: SECTION: RIDGE OF LABIAL PALP.
6μ. OBJECTIVE 7. X 143.
FIXED: FLEMMING + ALCOHOL. STAINED: BEST'S CARMINE.



3. TRANSVERSE SECTION: LATERAL WALL OF DIRECT INTESTINE.
6μ. OBJECTIVE 7. X 143.
FIXED FLEMMING. STAINED: MALLORY.



4. TRANS: SECTION. GILL FILAMENT. 6μ.
FIXED: ALCOHOL. STAINED: IODINE.



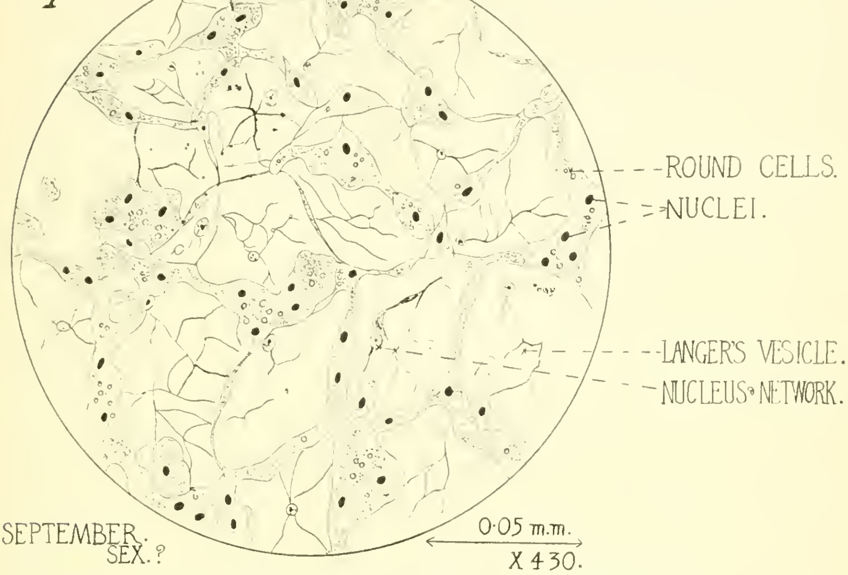
5. TRANS: SECTION. CONNECTIVE TISSUE OF MESOSOMA. 6μ.
FIXED: FLEMMING. STAINED: IODINE. OBJ: 1/12. X 483.

PLATE II

TRANSVERSE. SECTIONS: CONNECTIVE TISSUE OF MANTLE.

MYTILUS. EDULIS.

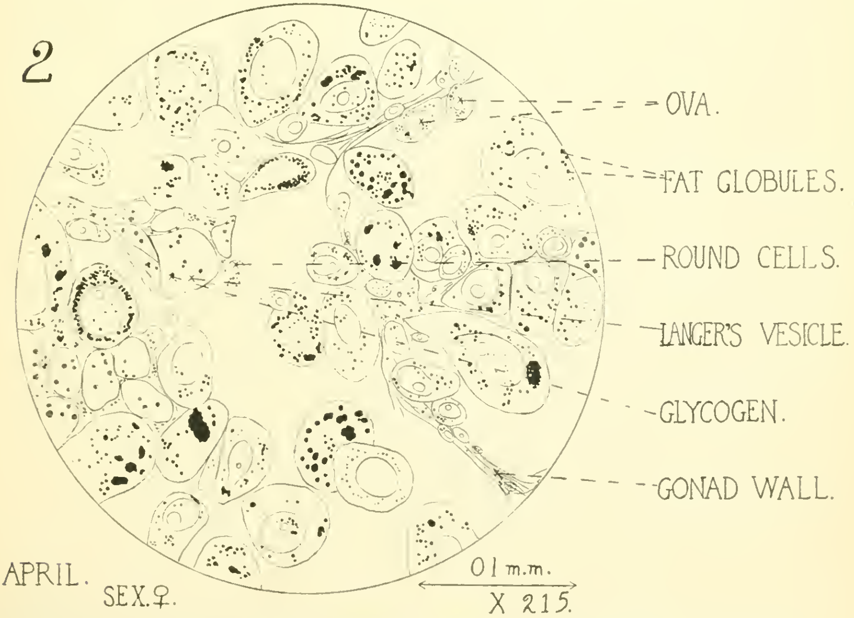
1



SEPTEMBER.
SEX. ?

SECTION 4 u: FIXED: CHROMO-NITRIC. STAINED: HAEMALUM EOSIN.

2



APRIL. SEX. ♀.

SECTION. 6u: FIXED: MOD FLEMMING+ALCOHOL. STAINED: BEST'S CARMINE.

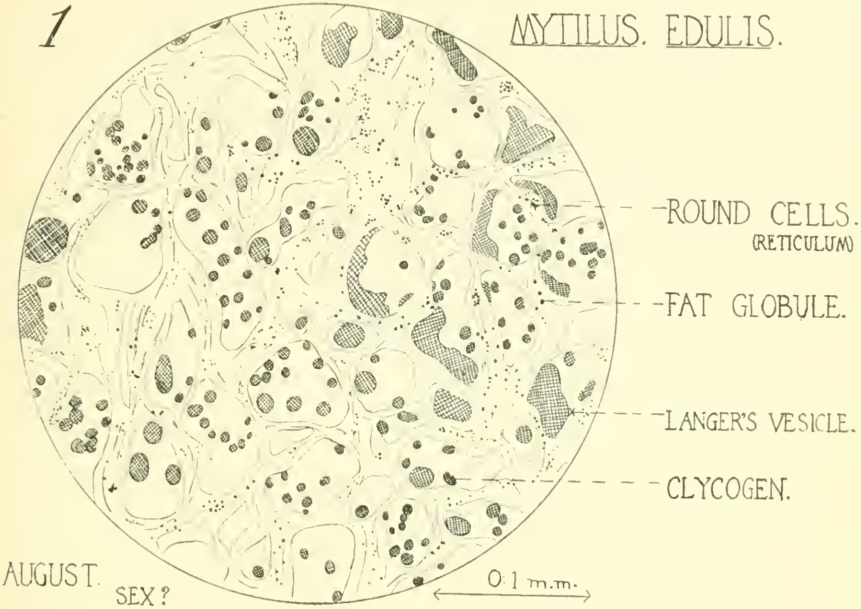
OBJECTIVES. 7.

PLATE III

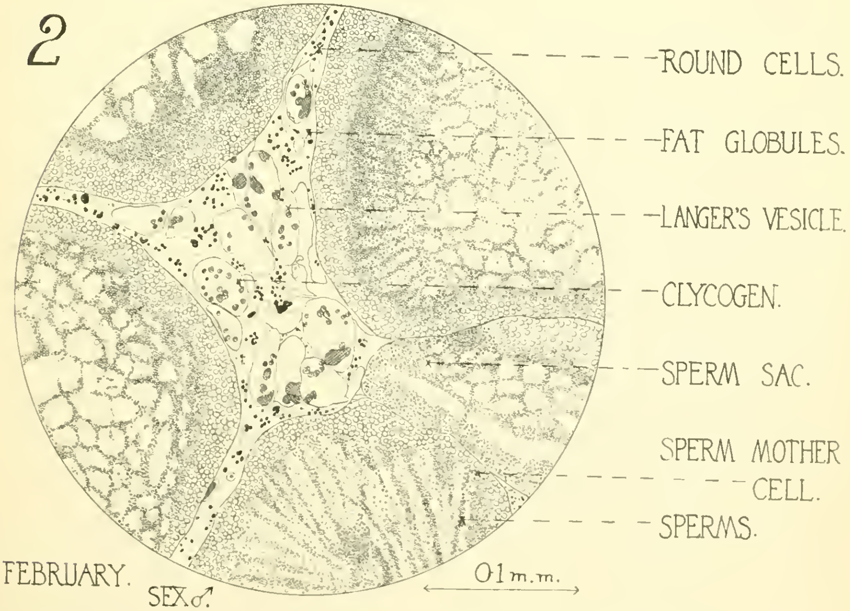
TRANSVERSE SECTIONS: CONNECTIVE TISSUE OF MANTLE.

MYTILUS. EDULIS.

1



2



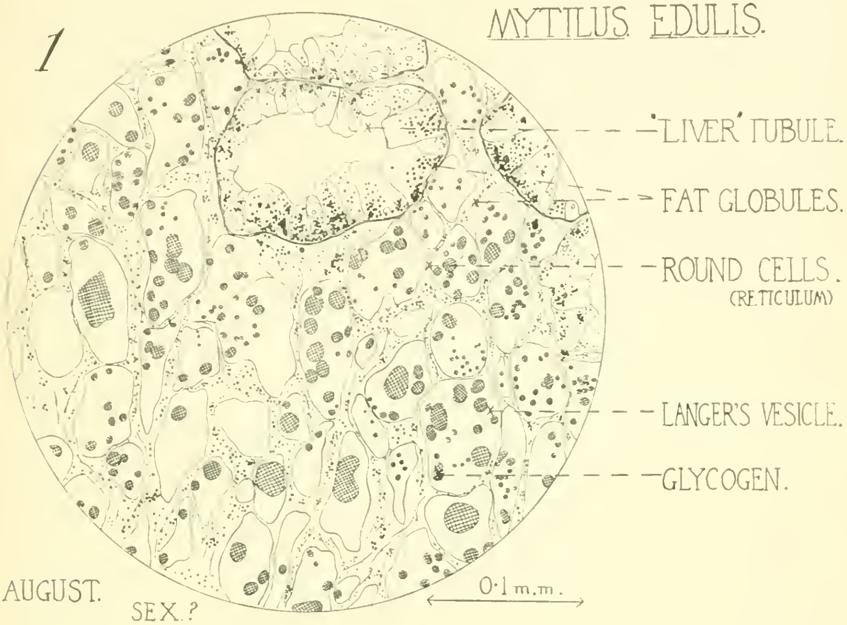
SECTIONS .6 μ : FIXED: MOD: FLEMMING+ALCOHOL. STAINED: BEST'S CARMINE.

OBJECTIVE. 7. MAG: 215.

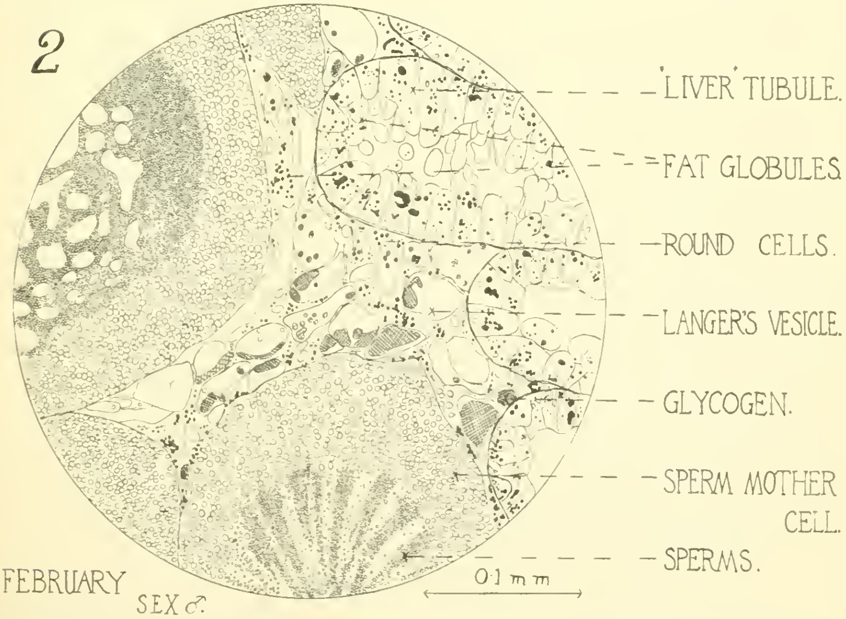
TRANSVERSE SECTIONS: CONNECTIVE TISSUE OF BODY.

MYTILUS EDULIS.

1



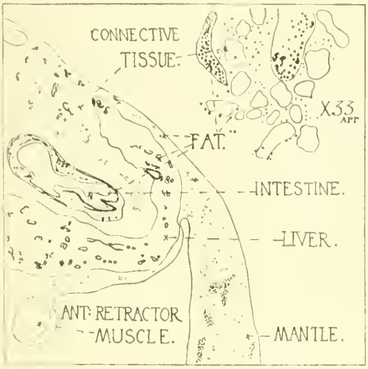
2



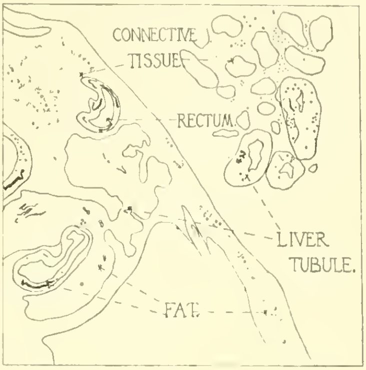
SECTION 6 *u* : FIXED. MOD: FLEMMING + ALCOHOL. STAINED: BEST'S CARMINE.
OBJECTIVE. 7. MAG: 215.

PLATE V

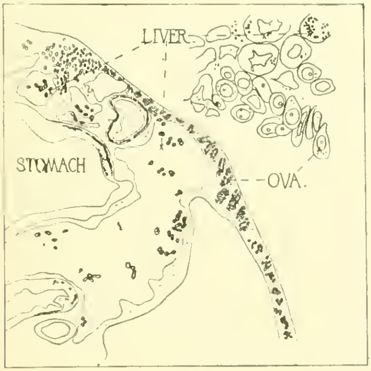
MYTILUS. EDULIS. TRANSVERSE SECTIONS. REGION OF GASTRIC GLAND. X. 5.



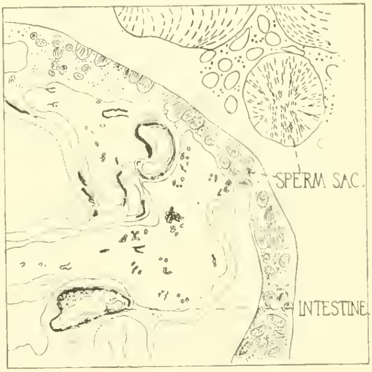
SEPTEMBER. SEX ?



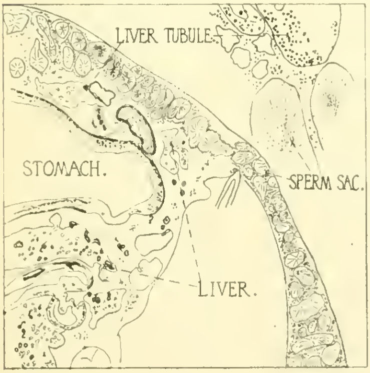
OCTOBER. SEX ?



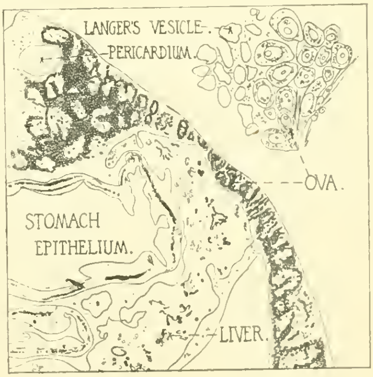
NOVEMBER. SEX. ♀



DECEMBER. SEX. ♂



JANUARY. SEX. ♂

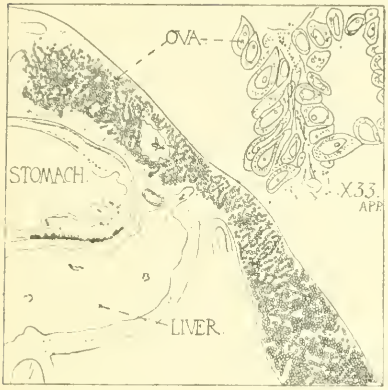


FEBRUARY. SEX. ♀

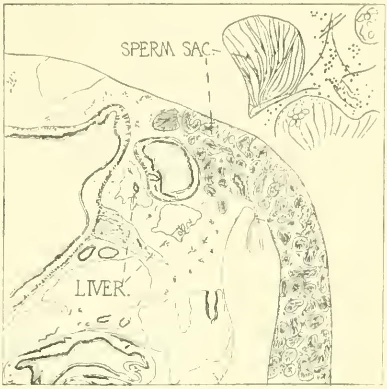
FIXED: 4%. FORMALDEHYDE. STAINED: SUDAN III. FAT REPRESENTED AS THICK BLACK LINES AND DOTS.

PLATE VI

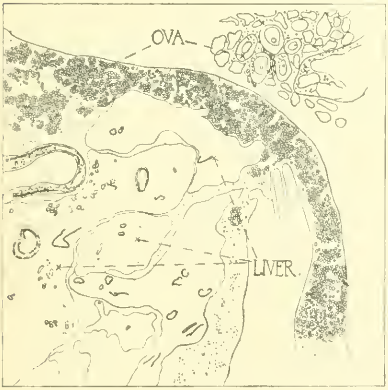
MYTILUS. EDULIS. TRANSVERSE SECTIONS. REGION OF GASTRIC GLAND. X.5.



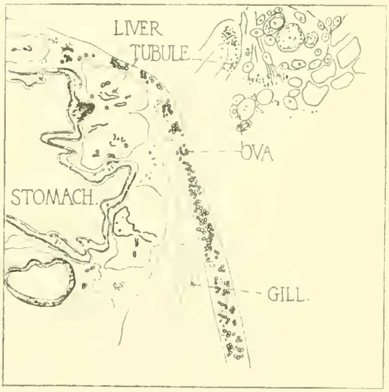
MARCH 2nd. SEX ♀.



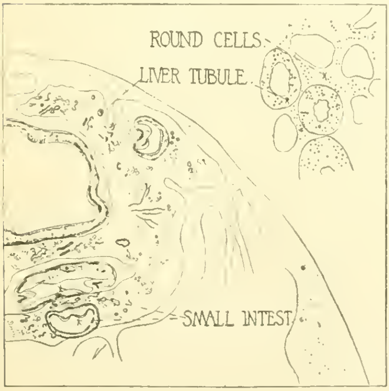
MARCH 22nd. SEX ♂.



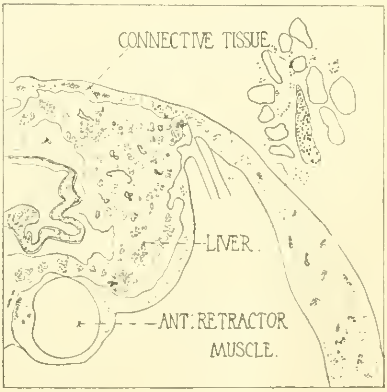
APRIL. SEX ♀.



MAY. SEX ♀. (SPAWNED)



JUNE. SEX ?.



AUGUST SEX ?.

FIXED: 4 % FORMALDEHYDE. STAINED: SUDAN III. FAT REPRESENTED AS THICK BLACK LINES AND DOTS.

Fig. 2. Appearance of ova lying in the reproductive follicles of a ripe female mussel. The connective tissue is almost entirely broken down, and there are spaces between the various ova.

PLATE III.

Fig. 1. The " round " cells of the connective tissue coalesce and form a reticulum, in which the cell boundaries are hard to define, particularly in sections of 6-8μ thickness. They have been represented, therefore, in this, and the following three figures, as a continuous network. The granular nature of these cells also has not been depicted, in order to bring out more clearly the distribution of the fat globules.

Fig. 2. Section of the mantle of a male mussel approaching maturity. The amount of connective tissue present shows a striking contrast to figure 1. The growth of the sperm sacs to the right of the field has actually caused a break down in the connective tissue cells.

PLATE IV.

Figs. 1. and 2. These show a similar contrast to the figures in Plate III. There is abundance of fat in the " liver " tubules as well as in the " round " cells, but the histochemical methods used demonstrate glycogen only in the Langer's vesicles.

PLATES V AND VI.

The figures in these Plates are intended to give a general view of the distribution of fat in sections prepared during different months throughout a year. They are all obtained from the region of the mussel lying between lines A and B marked in Plate I. figure 1. Sections of the digestive gland are drawn in outline, and individual tubules and blind sacs are only represented when they show a marked staining reaction for fat. Intense staining is shown by thick black lines or masses; where the staining of the fat is less intense, the position is marked by dots. The pieces of tissue drawn to a higher magnification in the right hand corner of each figure, give a clearer idea of the distribution of the fat globules in individual cells.' It will be seen that as the mussels approach maturity in April, there is an increase of fat in connection with the reproductive tissues. The globules are contained in the ova in the case of the female, concentrated about the sperm sacs in the male.

THE IRISH SEA COD FISHERY OF 1921-23

BY

JAS. JOHNSTONE, W. C. SMITH, AND R. A. FLEMING.

The cod fishery of the Irish Sea is relatively unimportant when compared with that for plaice, soles, skates and rays. The area is placed not far from the southern limit of economic distribution of the cod : thus the main fishery, such as it is, is practically restricted to the waters round Isle of Man and between there and the Cumberland coast and it is of very little significance in St. George's Channel south from Isle of Man. This, too, is about the northern limit of the great hake fishery, so that while the latter fish is taken to the south and west of Isle of Man it is rare that more than isolated specimens are caught to the north and east of the Island. Both in Isle of Man and off the Cumberland coast the cod fishery has little more than local importance. The fish themselves are, at the best, mediocre in quality, watery and tasteless when compared with the cod from the north-east coasts of Britain. The Manx and Cumberland cod are sold fresh and there is no curing industry.

The following account is based on observations made, mainly during 1922, and as part of a general scheme of investigation organised by the Ministry of Agriculture and Fisheries. It is incomplete in that some further attention is required in respect of the early stages in the life-history of the fish and scale-readings on the big scale have still to be made. The investigation has value in so far as its results can be utilised in the preparation of a general account of the life-history of the species in the British seas.

General Account of the Fishery.

In Manx waters the cod-fishery is prosecuted throughout the year in more or less degree but with increased intensity during the winter months, January-April. The smaller cod, up to about 60 cm. in length, are permanent inhabitants of these waters, and their numbers will probably be fairly constant during the year ; but on the approach of winter, with the lowering temperature of the sea, a great in-shore movement of large mature fish sets in. Indications of their approach are received in November, and the numbers then gradually increase, reaching a maximum in March. After this the shoals rather rapidly fall back, and the off-shore movement is usually completed by the end of April. During the early summer months the local cod are found in depths of from 6 to 30 fathoms, but in August they move in-shore and are caught close in to the rocks till November, when a movement out to deeper water takes place. The fish themselves are nearly always the ordinary grey cod, but in the summer months occasional specimens of " red cod " are taken.

Physical Features of the Area.

There are dissimilarities in the physical features of the fishing zones of the western and eastern sides of the Isle of Man, and these divide the fishery into two natural areas. Deep water, with a goodly proportion of mud in the bottom deposits, exists to the westward, while comparatively shallow water and sand characterise the eastern area. This affects the class of fish taken, and the run of cod on the eastern side appears to be larger than that encountered to the westward. The eastern fishing is carried on in the channel between Cumberland and the Isle of Man, the boats working 14 to 20 miles out from the Island at times. Depths here are not so great as to the westward, being usually between 18 and 23 fathoms, although 27 fathoms may be worked at the end of the winter fishery when operating to the north, between the Point of Ayre and the Scottish coast. On the west, deep water occurs comparatively close to the coast-line, and the bottom out to

30 fathoms is either sand or a mixture of sand and mud; over this depth it is mud.

Spawning.

The large winter cod are taken in great numbers along the Cumberland coast and all round the shores of the Isle of Man, except in the shallow north-eastern waters off Ramsey Bay. They come in to this region to spawn, and the gonads rapidly ripen during January and February, so that the majority of the fish are ready to commence spawning by the end of the latter month. March is the principal spawning month, and at that time the genital organs are functioning in the majority of the cod taken. Spent fish begin to appear in March and become more numerous as the season advances. About mid-April spawning is completed and the spent fish move off.

The summer cod are smaller and quite different in appearance to the spawning fish, being firmer in the flesh and much darker on the back, and most of them are immature, although large mature fish do appear amongst them.

Size.

In the winter fishery the largest cod are taken to the eastward of the Isle of Man, and the following table (which is compiled from measurements taken in March and April, 1922) gives the sizes of the fish landed at the different ports of the Island.

Peel and Port Erin represent the western fish, Douglas and Ramsey the eastern shoal, while the Point of Ayre cod are local fish taken by Ramsey boats at the end of the winter season, and they represent the run of cod caught during the summer months.

The mean size of all the spawning fish measured is 74·16 cm. with the mode at 76 cm. and half the total grouped between 66 and 79 cm. The frequencies diminish somewhat

Table I. Manx Cod, March-April, 1922.

L	1	2	3	4	5	6
32	1	...	...	...	...	...
33	1	...	...	...	...	...
34	1	...	...	...	...	...
35	...	...	...	...	...	...
36	...	...	...	...	...	...
37	1	...	...	...	...	...
38	...	...	...	...	...	...
39	...	...	...	...	...	...
40	...	...	...	...	...	...
41	3	...	...	...	...	...
42	4	...	...	...	...	...
43	6	...	...	...	...	...
44	10	...	...	...	...	...
45	21	...	...	...	...	...
46	23	...	...	...	...	...
47	44	...	...	...	1	1
48	40	1	...	...	1	2
49	32	1	...	1	...	2
50	48	3	...	...	...	3
51	46	2	3	...	...	5
52	32	3	2	...	...	5
53	21	7	3	...	...	10
54	21	15	6	2	...	23
55	11	7	3	3	2	15
56	22	23	2	6	2	33
57	24	20	7	6	1	34
58	37	22	8	8	1	39
59	41	21	22	9	2	54
60	37	39	33	11	1	84
61	52	40	27	17	2	86
62	41	55	22	11	5	93
63	33	38	25	19	3	85
64	36	53	28	13	5	99
65	35	48	33	12	3	96
66	21	62	44	17	2	125
67	25	64	45	20	2	131
68	24	59	38	21	3	121
69	9	63	43	22	7	135
70	14	87	47	21	7	161
71	14	60	51	28	4	143
72	11	89	55	23	4	171
73	15	77	45	17	6	145
74	10	93	54	29	3	179
75	11	83	42	18	3	146
76	8	97	64	23	2	186
77	11	78	34	33	4	149
78	7	74	34	31	5	144
79	4	58	45	16	3	122
80	5	51	33	18	3	105
81	6	47	34	21	4	106
82	7	50	28	17	1	96
83	6	43	40	25	2	110
84	4	26	35	30	2	93
85	5	17	20	22	...	59
86	3	19	26	16	2	63
87	5	18	24	21	...	63
88	2	18	15	16	...	49
89	5	18	17	19	...	54
90	2	16	13	21	2	52
91	3	4	15	12	1	32
92	1	7	16	16	...	39
93	...	5	10	18	1	34
94	...	2	7	12	...	21
95	...	2	7	11	...	20
96	...	1	6	12	...	19
97	...	1	8	4	...	13
98	1	3	5	7	...	15
99	...	...	6	4	...	10
100	1	2	4	3	...	9
101	1	...	2	3	...	4
102	...	1	1	3	...	5
103	...	...	1	1	...	2
104	...	...	...	1	...	1
105	...	...	...	...	...	...
106	...	...	...	...	...	...
107	...	...	1	...	...	1
108	...	...	...	2	...	2
109	...	...	...	1	...	1
110	...	...	1	1	...	2
111	1	...	1	...	...	1
112	...	...	1	...	...	1
	966	1,793	1,242	773	102	3,910

References to headings :—

L = Mean length in cms.
1 = Point of Ayre district.
2 = Peel district.
3 = Douglas district.
4 = Ramsey district.
5 = Port Erin district.
6 = Totals of 2 to 5.

2 to 5 represent the Manx winter cod fishery.

rapidly on each side of this range, so it is evident that the run of cod which come in to the Irish Sea to spawn is dominated by fish contained in a short range of sizes, pointing to a strong representation of one age group. On examining the figures from Peel, Douglas, and Ramsey separately, the higher frequencies of the eastern fish of the larger sizes (over 80 cms.) becomes apparent, and this is very pronounced at Ramsey, where the shortest half-range is lengthened out to 69-87 cm., indicating a much more even distribution of sizes in this area. A size curve drawn for the Point of Ayre cod (summer) shows two distinct cusps at 50 and 61 cm., and these evidently consist of two age groups, possibly II and III.

Quantity and Movements.

During the 1922 investigations it was found that March was the best month for the fishery: Ramsey boats then making the heaviest catches. Peel figures fluctuated very little during February, March and April, but the Douglas ones were small to begin with and gradually rose during March to reach a maximum in the fourth week of the month, and then dropping rather abruptly in April. Ramsey seemed to get the large quantities early in March and retained them during the month, experiencing a drop in April similar to Douglas. The constant figures at Peel were probably due to the fact that the boats were able to follow the fish to a greater extent than the other fleets, and so kept among the shoals during the greater part of the season.

These figures seem to suggest a rather important movement of cod from the north into the region between Cumberland and the Isle of Man early in March, the shoals reaching the southern waters off Douglas later in the month, and then retreating rather suddenly in April. The western cod would appear to move south till the end of March, afterwards returning to the north.

Food of the Cod.

Only a few observations were made. These refer to the contents of the stomachs of the fish examined in the Liverpool laboratory. They confirm other observations made in the past. The predominant food organisms identified are given, with the numbers of fish in which they occurred.

Galathea—10 fish.
Ophiothrix (various species)—9 fish.
Buccinum undatum—4 fish.
Whiting (mostly small)—3 fish.
Hyas coarctatus—3 fish.
Eupagurus—2 fish.
Sandeels—1 fish.

Parasites and Diseases.

A number of Copepod and Helminth parasites have been found and identified, and several cases of malignant growths and other diseases have been studied. A summary account of these conditions and parasites will be given later.

Life-History in General.

A number of observations have been made, of course, but the opportunities for an adequate study have not been given to us. Much has still to be done with regard to the occurrence of the eggs and pelagic larvae and the rate of growth has still to be deduced from a study of the scales. For some reason it is very difficult to " read " the scales of Irish Sea cod and the chances of error are apparently so large that this part of the work has been left aside in the meantime.

Measurements of the codling and small codling taken in the shallow waters are being made. We give only (in the meantime) a series of measurements of the fish sent us by the skipper of a Fleetwood prawn-trawler. These are characteristic.

Length ...	4,	5,	6,	7,	8,	9,	10,	11,	12,	13,	14,	15,	16,	17,	18,	19 cms.
August ...	1,	21,	30,	18,	14,	20,	12,	9,	2,	1,	1,	1,	1,	2,	3,	1
October ...						3,	2,	6,	9,	4,	19,	12,	6,	2,	0,	1
Nov.-Dec. ...						1,	1,	1,	8,	9,	8,	4,	8,	5,	2,	1

Small codling are hardly seen until the beginning of the autumn, when they are fairly abundant on the rougher kinds of sea bottom. They are about 5 to 8 cms. in length and have the characteristic "chess-board" pigment pattern. By the end of the year they have attained an average length of about 15 cms. The little table given above represents, then, the O-group of cod in local waters.

The "Condition" of the Fish.

A good series of samples of the cod caught at Port Erin were obtained by Mr. T. N. Cregeen and sent to the Liverpool Laboratory. There the fish (2 in each sample) were weighed and measured, the gonads and livers were taken out and weighed and the stomach contents were examined. The length-weight coefficient k was calculated from the formula $weight = k \times (length)^3$ but k in the results is multiplied by 100. The weights of the gonads and liver are represented as ratios, weight of fish *divided* by weight of gonads or liver, as the case may be. Thus the result 108 means that the weight of the fish is 108 times greater than the weight of the gonads and so also with the weights of the livers. These weights, ratios, lengths and coefficients are given in Table II.

At the same time rough chemical analyses were made. A fillet was cut from each fish and slices of this were taken so that a sample representative of the flesh of the whole fish was obtained. The weight of each sample of flesh (representative of two fish) was about 8 to 10 grams. A similar sample of the livers was taken. Each sample was dried at 100° C till the weight was constant. The dried material was then

Table II. Port Erin Cod, Seasons October, 1921, to March, 1923.

Date	Male					Female				
	Length	Weight	Coefficient *k*	Weight / wt. gonads	Weight / wt. liver	Length	Weight	Coefficient *k*	Weight / wt. gonads	Weight / wt. liver
26.10.21	...	...	...	...	...	68	2,850	·904	219*I*	38
	...	...	...	...	...	69	3,163	·963	320*I*	55
25.11.21	59	2,289	1·114	108*I*	40	57	2,157	1·164	308*I*	33
14.12.21	70	3,760	1·096	87*M*	31	82	5,750	1·042	61*M*	22
14.1.22	65	3,420	1·170	16*M*	40	70	3,496	1·019	103*M*	76
1.2.22	...	...	...	...	...	67	3,365	1·119	54	35
	...	...	...	...	...	66	3,066	1.067	255	66
15.2.22	72	3,970	1·063	15*M*	81	67	3,252	1·081	361	36
9.3.22	71	3,982	1·112	19*M*	54	...	...	...	...	...
	71	4,025	1·175	14*M*	32	...	...	...	...	...
23.3.22	...	...	...	...	...	74	4,670	1·152	8*M*	28
	...	...	...	...	...	70	3,720	1·084	7*M*	40
5.4.22	...	...	...	...	...	64	3,080	1·175	14*M*	40
	...	...	...	...	...	65	2,410	·878	80*M*	86
4.5.22	...	...	...	...	...	61	2,380	1·059	297	57
	...	...	...	...	...	66	2,793	·972	215	25
19.5.22	...	...	...	...	...	66	3,270	1·138	363	33
31.5.22	...	...	...	...	...	64	2,560	·976	*B*	...
	...	...	...	...	...	68	3,310	1·050	*B*	...
21.6.22	84	6,210	1·105	*S*	16	...	...	...	...	...
	84	6,520	1·100	225*S*	...	...	...	...	...	...
20.9.22	...	..	...	...	...	62	2,442	1·027	222	41
	...	...	...	...	...	60	2,099	·971	233	31
7.10.22	65	2,580	·939	516*B*	32	...	...	...	...	...
	61	2,310	1·192	*B*	...	...	...	...	...	...
27.10.22	...	...	...	...	...	59	2,200	1·071	183*B*	50
	...	...	...	...	...	60	2,197	1·017	*BI*	...
11.11.22	63	2,531	1·012	281*I*	68	61	2,520	1·110	360*I*	74
23.11.22	...	...	...	...	...	63	2,520	1·081	115	93
	...	...	...	...	...	61	2,380	1·049	238	57
5.12.22	62	2,409	1·001	343	78	...	...	...	...	...
	66	2,631	·903	263	61	...	...	...	...	...
22.12.22	62	...	...	...	...	61	2,690	1·185	117	43
8.1.23	70	3,880	1·131	23*M*	25	68	3,594	1·142	53*M*	31
1.2.23	...	...	...	...	...	68	3,740	1·119	39	27
	...	...	...	...	...	65	2,696	·892	225	82
14.2.23	70	3,705	1·080	17*M*	45	...	...	...	...	...
	68	3,310	1·053	27*M*	58	...	...	...	...	...

NOTES :—*B* means "bad condition"; *I* "immature"; *M* "mature"; *S* "spent."

extracted in a Soxhlet apparatus, using carbon tetrachloride as the solvent. About 0·5 grm. of the extracted material was taken for a nitrogen estimation by the Kjeldahl method and the rest was ignited so as to get a value for the percentage of non-volatile matter, or ash. The results of the analyses are given in Table III. The experimental error may be taken as not greater than ± $\frac{1}{4}$ per cent.

The Measurements.

Consider first the length-weight coefficient k. This, we expected, would vary in much the same way as it does in the case of the plaice. When the temperature of the sea is low (in the months December to March) the value of k drops to about 0·9 (or even less) and when the temperature is highest (in the months June to September) k rises to 1·1 (or even higher). The difference is due to the nutrition of the fish: in the winter months they do not feed and the body becomes thin to quite a noticeable degree. As the temperature rises in April the plaice begin to feed voraciously and become plump, with the result that k rises to its maximum values. All this applies to immature fish with ovaries and testes that do not vary much in mass.

Taking the female cod measured and weighed in Table II, we do not find this seasonal variation in the coefficient k—or, at least, it is not at all obvious. Thus:

January to March ... ... k = 1·075
April to June ... ... k = 1·075
July to September ... ... k = 0·999
October to December ... ... k = 1·058

The mean for the whole year is 1·054.

Where there are differences in k these appear to be due to the effect of spawning on the cod. Consider the female cod of 70 cms. long and of 3,720 grams in total weight of date

23.3.22 : its ovaries weighed 555 grams and its k was 1·084. Suppose that, all at once, it spawned and its gonads shrivelled up to only a few grams : its length would still be 70 cms. but its weight would decrease to about 3,200 grams and its k would fall to about 0·92. Looking over the table, however, we find spent fish in which the k is greater than the above example would lead us to expect. Thus on 1.2.22 and 15.2.22 there are cod in which the total weights are 255 and 361 times the weights of the gonads and these were probably fish that were partially spawned or spent. Yet their k's were 1·067 and 1·081 and not the approximation to 0·92 that we might have expected.

It is very probable that spawning is a rather protracted act and that while it is in progress the fish is feeding greedily. New tissues are thus being formed at such a rate that the loss of weight due to the extrusion of the eggs or milt is compensated.

Consider next the variation in the mass of the liver. In Table II this is given in the last columns, where the entries show how many times the total weight of the fish is greater than that of the liver. Thus we get (male and females taken together) :

January to March	46
April to June	43
July to September	36
October to December	55

This difference in the weight of the liver throughout the season is much less than was expected. It was believed that, just as a notable quantity of fat is stored in the connective tissues of the bodies of herring during the pre-spawning period so fat would be stored in the livers of mature cod, during the same phases. This is the case, to some extent. The liver is relatively big during the months October to March and relatively small during the months April to September. But the variations are very much less than were expected.

The Analytical Results.

The data of a series of routine estimations are given in Table III. The following results are to be noted :—

Table III. Port Erin Cod, Seasons, October, 1921, to March, 1923.

Date	Muscle Substance				Date	Liver			
	Water	Fat	Ash	Nitrogen in dry substance		Water	Fat	Ash	Nitrogen in dry substance
25.10.1921	81·5	0·1	0·9	14·7	25.10.1921	47·3	40·5	0·5	12·0
25.11.1921	80·5	0·02	0·9	15·3	25.11.1921	37·2	43·1	1·0	11·1
15.12.1921	81·2	0·2	0·5	16·0	15.12.1921	32·6	53·8	0·6	11·7
14.1.1922	81·6	0·2	0·8	15·3	14.1.1922	39·7	44·1	0·7	11·7
1.2.1922	81·0	0·3	0·9	15·3	1.2.1922	59·1	23·3	0·7	12·8
15.2.1922	82·0	0·2	0·9	15·3	15.2.1922	45·5	41·6	0·5	12·3
8.3.1922	80·3	0·2	1·1	14·9	8.3.1922	32·7	49·0	0·9	12·1
22.3.1922	83.5	0·2	0·7	15·7	22.3.1922	53·0	32·0	0·9	12·9
5.4.1922	82·2	0·1	0·9	15·8	5.4.1922	49·0	39·2	0·6	12·8
4.5.1922	81·0	0·3	0·9	15·8	4.5.1922	41·3	45·5	0·6	9·5
19.5.1922	80·8	0·1	0·8	15·6	19.5.1922	41·9	46·7	0·7	18·5
31.5.1922	80·9	0·1	0·7	17·0	...	...	...	...	...
21.6.1922	81·3	0·1	0·7	17·1	21.6.1922	38·3	51·2	0·5	15·3
20.9.1922	80·8	0·1	0·8	17·0	20.9.1922	41·7	47·0	0·4	18·4
7·10.1922	81·5	0·04	0·7	16·9	7.10.1922	48·2	44·1	0·3	18·5
27.10.1922	81·5	0·1	0·7	16·8	27.10.1922	31·8	59·6	0·3	18·6
11.11.1922	81·9	0·2	...	...	11.11.1922	64·9	18·5	0·3	18·4
23.11.1922	81·9	0·1	0·7	16·6	23.11.1922	71·8	11·3	0·7	18·5
6.12.1922	81·7	0·3	0·6	17·5	6.12.1922	57·2	26·1	0·8	18·6
22.12.1922	81·5	0·2	0·7	15·7	22.12.1922	67·1	16·4	0·6	17·7
18.1.1923	81·1	0·2	0·7	15·5	18·1.1923	35·4	47·7	0·8	17·9
1.2.1923	82·1	0·2	0·8	16·1	1.2.1923	52·5	34·2	0·7	17·5
14.2.1923	81·1	0·2	0·8	16·2	14.2.1923	50·4	37·3	0·4	17·9

(1) *The large quantity of water in the flesh.* This varies closely round about 81 per cent. The extremes are only from 80·3 to 83·5. No apparent variation with the seasons can be noted.

(2) *The small quantity of "fat" in the flesh.* This is, as a rule, 0·2 per cent. of the wet tissue. Even those small quantities estimated are hardly to be regarded as true "fat" and may represent "extractive" substances of quite another nature.

(3) *The "proteid."* The usual routine was, at first, followed. The nitrogen found in the residue obtained by first drying and then extracting the muscle substance was multiplied by 6·25 so as to get proteid. The results were unsatisfactory, inasmuch as in many cases the totals of water, "fat," ash and "proteid" came to considerably more than 100 per cent. Obviously the factor 6·25 ought not to be used and some other method of estimating the proteids present in the muscle substance must be found. So the tables give only the percentages of nitrogen found in the dry, fat-free, ash-free substance. These values would show any decided seasonal variability, if such exists. They show nothing of the kind, however; the flesh was poor in nitrogen during the winter and spring of 1921-2 and much richer in the summer of 1922 and the winter of 1922-3.

(4) *The liver.* Here again no marked seasonal variability in composition is to be seen. There are quite notable fluctuations in the quantities of water and fat found, but these are apparently "accidental"; that is, they are not to be traced to any one predominant cause of variability but to a number of independent ones. Obviously there is a considerable error of sampling: one cod may differ from another, in respect of the nature of its liver, in a remarkable degree. Thus the fat in the organ varies between 11 and 60 per cent. but usually is about 40 to 45, and there are compensatory variations in the

percentage of water. It is clear, however, that the percentage of nitrogen in the liver substance was remarkably low during the winter and spring seasons of 1921-2 and was much higher in the summer of 1922 and the winter and spring of 1922-3. The variation is concomitant with that of the nitrogen in the muscle substance, but it is greater. That is all one can say.

Evidently the winter of 1921-2 was an unfavourable one in that the cod were not so well nourished as they appear to have been during the succeeding season.

Conclusions. What is quite clear is that there is no such definite seasonal variability in the metabolism of these Manx cod as one finds in the herrings from the same district. The habits of the fish are seasonal and the shoals come and spawn regularly in the waters round the Island. But this is not a typical cod-fishing ground ; it is near the southern limit of distribution of a fish which has an extreme northerly habitat, and so one may expect to find that the seasonal rhythm of metabolism is not so clearly indicated here as it is in the Norwegian region, for instance. The observations made with respect to the Irish Sea cod may therefore have much value when they are compared with similar investigations made on more typical cod grounds.

SCALE INVESTIGATIONS OF SHOALING HERRINGS FROM THE IRISH SEA.

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The samples of herrings investigated are those which have been taken during the years 1920, 1921, and 1922 in the Irish Sea, off the Welsh, Manx, Lancashire and Cumberland coasts. They were all taken in drift-nets except two samples from Menai which were taken in the fish weirs in the Straits. Until more evidence is available for the more exact determination of the period when the growth of the scale of the Irish Sea herring commences and when this growth is checked, it has been assumed that growth commences at the beginning of May and continues until the beginning of September when retardation of growth sets in.

Einar Lea, in his report on "Age and Growth of Herrings in Canadian Waters" (1918), suggests that growth of the small Norwegian herrings becomes apparent at the end of April and ceases some time during September. This seems to bear out our own observations, which are, unfortunately, limited in the case of the herring, but very extensive in the case of plaice.

It should be remembered that autumn and winter spawned herrings exist for about the first six months of their life without scales, so that the first area on a scale is laid down during the period May to September, which we consider to be the scale-growing period. The naked period is not indicated on the scale and should not be overlooked when estimating the actual age of the fish. A winter ring only becomes definitely a ring when it is bounded by a summer growth of an appreciable extent, so that by the time this ring (in a one-ring fish) is surrounded by a summer growth the fish is well on the way towards completing two years of life; similarly with older scales the actual age in years is reckoned as being one more than the number of winter rings on the scale.

To help us to clear up these questions the otoliths of all the herrings taken during October, November, and December, 1922, were examined. The expectation was that the recently formed opaque summer ring would be found to be surrounded by a clear winter ring, but this was not fulfilled. As with the scales, so with the otoliths ; it seems to be necessary for the clear winter ring to be surrounded by a summer area before it can be definitely made out as a winter ring. In some cases it almost looked as if the summer area were surrounded by a clear winter growth whilst in others the summer area seemed to continue to the edge. In any case the thinness of the otolith at the edge makes it extremely difficult to say definitely whether it is opaque or transparent. One fact, however, was revealed. When scales and otoliths were compared, the summer growth areas of each were found to agree. Suppose a scale showed two winter rings and three summer areas, then the otolith would show two clear winter rings, three summer opaque rings plus the transparent nucleus. The transparency of this nucleus strengthens the assumption that *herrings having otoliths with transparent nuclei are autumn and winter spawned.* Young plaice spawned in the spring, with all the summer before them form an *opaque* nucleus and then the clear ring which surrounds it during the following winter. Evidently the conditions which determine the formation of clear and opaque rings are the same for each species.

Every sample of Irish Sea herrings examined showed the otolith nucleus to be transparent. It remains to be seen if this holds good for spring spawned herrings.

Considerable difficulty has been experienced in counting scale rings when they become numerous. Up to five no particular difficulty is encountered, but when they exceed that number they are generally packed so closely together that it requires great care to separate and count them. When they amount to eight or ten rings the winter ring is almost as wide as the summer growth, so that any biometrical investigation of scales becomes of questionable value when applied to Irish Sea herrings with more than five rings. In the case of otoliths the same difficulty arose, due to crowding of the rings, although in a few exceptional cases of eight and nine ring scales we got excellently formed otoliths in agreement. It did not follow that when a scale was particularly well or badly ringed, or in any other way abnormal, the otolith would correspond. Often a scale was very clearly ringed and the otolith was unreadable or *vice versa*.

Results of the examination of various samples.

It has been considered preferable to analyse each sample individually rather than aggregate as one sample a season's catch from any of the respective localities. By doing this we can show the occasional sudden changes in the age and stage of maturity composition of samples, which would otherwise be lost if treated as one sample.

Wales (see Table I and Fig. 1).

In October, 1921, the bulk of the sample was made up of 1918 spawned fish, that is, three year olds. They were mostly "full," a few being in the earlier stages or "spent." The next group in importance was the group spawned in 1917, mostly "full" with some "spents."

The occurrence of two year old fish is of interest, as this is the only sample in which they occur. Although only forming 8 per cent. of the total sample the wide range of their condition of maturity is somewhat remarkable, being from Stage I to V, though the majority are in the earlier Stages, I, II, and III. The remainder of the sample is made up of older fish, five to twelve year olds, which were all "spents," except one fish, eight years old, in Stage V. This sample was taken from fish landed at Aberystwith, Aberdovey and Barmouth during the last week of October.

The sample of December 22nd, 1921, was taken in the Menai fish weir in the Menai Straits. It was made up mainly of fish spawned in 1918, 1916, 1915, and 1914. The 1917 year class was not well represented and the 1919 group was entirely absent. Most of the fish in this sample were full (Stage V). There were odd recent spents in Stage VII, and a few presumably recovering spents in Stage II.

The sample of January 20th, 1922, was taken in the same fish weir in Menai Straits and was quite different in character from the previous sample. There were two main groups, the 1918 and 1917 classes. Older fish were represented by two individuals only, 1915 and 1914. They were all in the early stages, mostly II, and were probably recovering, except the single 1914 fish which was full. The 1915 fish was in Stage II and also probably recovering.

Analysing the samples taken during the end of 1922 in Cardigan Bay off Newquay and the sample from Aberdovey we find that, with the exception of those fish taken on November 15th and December 5th, they were mainly composed of four and five year old fish, 1918 and 1917 year classes, mostly in Stage V. One striking exception, however, was the sample of October 17th. Most of these fish were 1918 and 1917 year class, 1918 predominating, and they were all spent except the single 1919 fish which was full. The two exceptional samples of November 15th and December 5th were made up of older fish. In November the bulk of the fish were 1917 and 1916 year classes in Stage V. In December the 1916 and 1915 year classes predominated and varied from Stage V to VII. In each sample the 1918 year class was represented only by a few examples, which were in Stage V.

Comparing these two seasons, 1921-22 and 1922-23, it appears that the bulk of the fish caught in the 1921-22 season were the 1918 year group. The 1917 year group in two of the three samples was next in importance. The appearance of the 1919 year group was noticed for the first time. During the season 1922-23 the 1918 year group still predominated, followed by the 1917 year group. The 1919 year group now seemed to be well established, as they were present in all the samples but one on December 5th, 1922. Very little information has been gathered from the other year groups beyond the fact that the more aged the fish are, the less important they become in the composition of a sample, and it would be unwise to draw any conclusions concerning them owing to the smallness of the data.

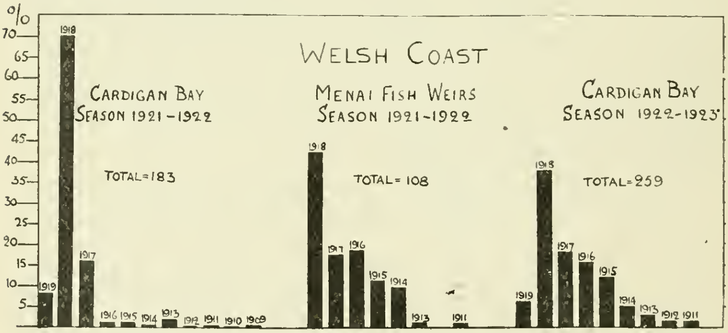

FIG. 1. Showing the percentage of each year class in the total seasonal samples from the Welsh coast. Cardigan Bay and Menai Straits fish weirs are shown as separate areas for the season 1921–1922.
The predominance of the 1918 year class is clearly shown.

The variation in maturity of the different samples is noteworthy when age and maturity are considered together.

In October, 1921, we found the 1919 year group to be mainly virgin fish in the early stages, I to III, with odd individuals full (IV or V). The 1918 and 1917 year groups were mainly full, IV or V, with odd individuals in the early stages, I, II or III, actually spawning, VI, or pent, VII. This suggests that maturity might be reached at the end of two years but more likely at the end of three.

With regard to the older year groups, these were mostly spent in October, " fulls " and spents in December.

In October, 1922, we found the 1919 year group to be *all* in Stage V, apparently having progressed during the year from Stage I, II or III. The 1918 and older groups were all represented in Stage V or VII, indicating that during the year they had filled up again and were ready to spawn or had already done so.

Isle of Man (see Table II and Fig. 2).

These samples were mostly taken in the deep water to the south-west of the Calf of Man. Investigation showed that in June, 1920, the bulk of the sample belonged to the 1917 and 1916 year groups in Stages I, II or III. In September, 1920, the two samples of the 9th and 22nd were a fairly equal assortment of 1915, 1914 and 1913 year groups, mostly in Stage V. The 1917 and 1916 year groups were present in the first of the two samples in appreciable numbers, but in the later sample were greatly outnumbered. In March, 1921, the sample was evenly made up of 1917, 1916, 1915 and 1914 year groups in Stage II. In May, 1921, the sample was much the same as in March both in year groups and maturity, but this sample is of particular interest inasmuch as the appearance of the 1918 year group was noticed for the first time—all in Stage II.

In August, 1921, the 1918 year group predominated, but now in Stage IV and V. The older groups, 1917, 1915, 1914 and 1912 were still represented, but 1916 and 1913 were absent.

In September, 1922, the appearance of the 1919 year group was observed, all full or spent, the 1918 year group being well established. The older groups, 1917, 1916, 1915, 1914 and 1913, were represented in more or less irregular proportions. The 1917, 1916 and 1915 year groups, however, made up the bulk of the three samples.

A comparison of the three years' samples reveals only one main fact. In June, 1920, the bulk of the fish were three and four year olds in the early stage of maturity. In September these fish were still present, but the shoals seemed to have been reinforced by older fish. All these fish were due to spawn or had already spawned. In March and May, 1921, the same year groups, now all spent and recovering, still remained in the vicinity. In August, 1921, they were still there and getting ready to spawn again. The 1918 fish, however, in May joined these older fish and were maturing, and by August were due to spawn and formed an important proportion of the sample. The older fish, however, still remained. No further data were available until the following September, 1922, when it was found that the 1918 year group had become established along with the 1917 and 1916 and older year groups and were all due to spawn or had already done so. The new group, 1919, as expected, had now joined and become a minor part of the shoal of variously aged fish. They were either full or spent.

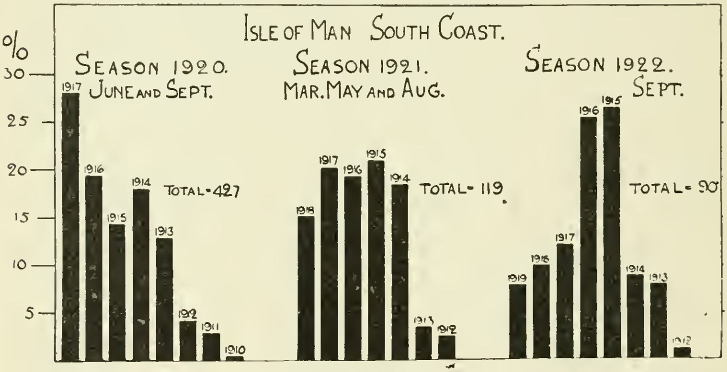

FIG. 2. Showing the percentage of each year class in the total seasonal samples from the Manx coast of 1920, 1921 and 1922, and the greater heterogeneity in age composition of the shoals than of those from Wales.

This seems to indicate that every year these herrings are reinforced by three-year old fish, the four, five and six, or even seven year olds, however, forming the bulk of the fishery in varying proportions. That these fish may remain in the vicinity of the Manx coast without going any great distance away seems to be shown by these facts.

Cumberland (See Table III and Fig. 3).

The first sample is one of eight fish only from Maryport. These were taken in November, 1921. They were all spent, and fish four to eight years old. On November 29, 1922, a good sample was received from Maryport. The fish were very mixed. The predominant year groups were 1916, 1915 and 1914, mainly recently spent, a few being still full. The 1919 and 1918 year groups were represented by a few individuals either full or recently spent.

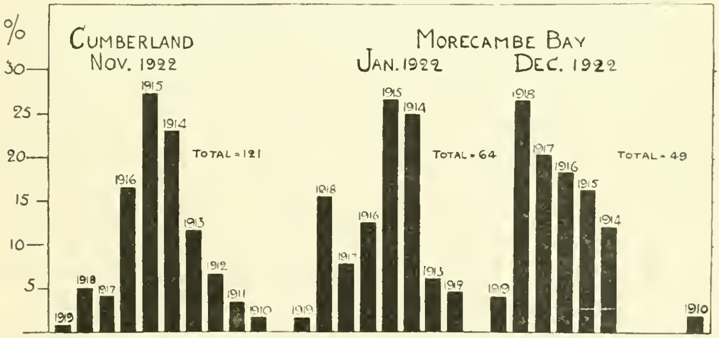

FIG. 3. Showing the percentage of each year class of the total seasonal samples from Cumberland and Morecambe Bay.

Lancashire (See Table III and Fig. 3).

On January 14th, 1922, a small sample of 18 fish from Morecambe Bay was examined and found to be a fairly equal assortment, comprising 1918 to 1912 year groups. The odd examples of 1918 and 1917 were full and the remainder spent or actually "running." On January 24th, 1922, a sample of 46 fish from Morecambe Bay was examined and was found to be very similar to that of January 14th. The 1915 and 1914 fish made up the bulk of the sample, although the 1918 group was not far behind. The presence of a 1919 year group was recorded. These fish were in the early stages and were probably recovering spents. On December 8th, 1922, a sample of 49 fish from Morecambe Bay was examined and found to be evenly made up of the 1918 to 1914 year groups, the 1918 year group predominating. The 1919 year group, although present, was not strongly represented. They were all spents except one fish in the 1919 year group which was in Stage IV and two in the 1918 year group in Stage V.

These results, although inconclusive, are encouraging. They indicate some definite lines to be followed in a more intensive investigation which can only be accomplished by continuous and extended sampling of the fish caught in the various localities. For example: the full herrings caught during summer off the Manx coast are from shoals which are mainly made up of a fairly equal assortment of from three to seven year old fish. Later in the year, during the winter, we find a similarity in the age-group composition of spent herring shoals taken off the Cumberland and Lancashire coasts. Are they the same shoals as those which were round the Manx coast during the summer?

The herrings taken off the Welsh coast in 1921 and 1922 were mainly 1918 year class fish. The older groups, though present, were not so well represented. Now in each year, 1921 and 1922, these 1918 herrings were mostly full. We might reasonably consider them to be virgin fish in 1921, that is, when they were three years old, but can we consider them so a year later when they are four years old? If they are, then it indicates that maturity may not be attained until four years of age. If they are not virgin fish, then it might indicate either that these fish, after spawning in 1921, reappeared in 1922 in the same localities, perhaps without making any extensive migration, or that they are fish which have come from some more distant waters. These points seem very obscure and only fuller investigation will throw any light on them.

Somewhat different facts are recognised when the Manx herrings are examined. We find the shoals are made up of a greater number of older fish without any marked predominance of any particular year class. The young virgin fish are present but do not form the same proportion as in the Welsh shoals. These young fish may have spent their early life in the Manx coastal waters or have migrated from either the Welsh, Irish or English waters to the spawning grounds—not yet definitely located—to the south, south-east or south-west of the Isle of Man.

The greater number of aged fish in the Manx shoals might indicate that the migrations of herrings, at least in the Irish Sea, are not so extensive as might be imagined. We have caught these aged fish in March, May, August and September in the same locality. In the early part of the year they were in poor condition and were recovering spents. Later they were full fish ready to spawn or already spawning—still in the same locality. A careful examination of both scales and fin measurements has failed to separate them into races. Furthermore, still later in the season, when the Manx fishery had finished we found large shoals of spent herrings of a similarly mixed year-class composition in the Cumberland and Lancashire coastal waters.

To establish a more definite connection between these fish of different localities we therefore propose to investigate on a much greater scale and collect more data. Among other points to be settled is the earliest age at which these herrings become mature. We have found an odd case where a herring had attained Stage V at the end of the second year.

This was from a sample of Welsh herrings. Altogether we found 15 fish at the end of the second year. Of these seven were in Stage I, four in Stage II, two in Stage III, one in Stage IV and one in Stage V. This we consider unusual, as we find all stages represented except actual spawning and spent in a group of two year old fish.

The practice of estimating the age of a herring by scale rings, while now generally admitted as being correct, is still criticised from a mathematical point of view. If young herrings move in homogeneous shoals up to their time of spawning, and we are inclined to believe they do, we certainly think they do not after spawning. We have definite information that the attainment of maturity is not uniform, that is, one three year old may be ready to spawn at a certain time whilst another may not be ready until some time later.

Now as a fish becomes ripe for spawning it seems reasonable to suppose that it must stop for a short time on the spawning ground. This means that it leaves its original shoal and there encounters fish of all ages which have gathered on the spawning grounds. As spawning is completed it moves away and joins other fish also leaving, and now becomes a member of a shoal of spent fish of mixed ages.

A Mathematical Criterion?

Here we may draw attention to a paper by Miss Sherriff, preceded by an introductory note by Professor D'Arcy Thompson*. The conclusions reached by these authors appear to be that a sample of herrings taken at random from a shoal of fish is an homogeneous sample. The shoal itself is regarded by Professor Thompson as an assemblage of fish "all of an age, fish that were spawned together, and that have ever since lived and swum and migrated together."

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* Herring investigation, Report on the mathematical analysis of random samples of herrings. Fishery Board for Scotland, Scientific Investigations. 1922. I.

The fish may vary in length from, say, 21 to 30 cms. and they may have scales that show, say, from three to a dozen rings : nevertheless they are of the same age and the differences in length and in number of rings on the scales are supposed to be due to variability in growth and in deposition of the substance of the scales. This conclusion follows, it is contended, from the form of the frequency distributions in respect both of length and number of rings. These distributions are "chance" ones. "The deviations from the mean, or predominant type, appear to follow the 'laws of chance,' the mathematical laws which govern the phenomena of 'variation' or 'variability.'"

The matter would be different, Professor Thompson contends, in the case of a sample of plaice taken at random. There we can sort out the fish into groups by mere measurement, to say nothing of the inspection of the number of rings in the otoliths, which tends "to confirm and define the several generations."

Miss Sherriff takes several samples of herrings arranged in groups by inspection of the rings on the scales. She calculates frequency curves by Pearson's method and shows that a Type I curve usually fits the empirical observations. Thus in one case a sample of 290 herrings consists of fish that have from 4 to 16 rings on the scales. The most frequent number is seven, and this is taken to be the age of the fish in the sample. The fish that have less than seven, or more than seven, are variants. They belong to the year-class seven, but some have more and others have fewer rings just through variations in the manner of growth of the scales. The variability from the normal number of seven rings follows the laws of chance. The fact that a Type I Pearson probability curve represents the facts with a good "fit" is said to show that the conditions are due to chance.

We have to acknowledge our indebtedness to Professor

Johnstone, who has very clearly indicated to us the mathematical reasoning and methods of the following discussion. As he points out, " we have to consider what Karl Pearson and his pupils mean by ' chance ' in this connection. All distributions of the frequency of occurrence of some measurable and variable characteristic in a group of animals that are homogeneous with respect to that characteristic can be represented by a certain differential equation, which is

$$\frac{1}{y}\frac{dy}{dx} = \frac{x - a}{c_0 + c_1x + c_2x^2}$$

When this equation is solved so as to give the corresponding frequency expressions there are three principal forms.

" (1) The constants c_1 and c_2 vanish, leaving c_0, and Type VII, the Gaussian curve of error, is obtained. The variation from the modal value of the character is due to the operation of a great number of small factors. All these are of equal valency ; they are independent of each other and each factor contributes with equal frequency results in excess or in defect of the mean. The curve is symmetrical.

" (2) The constant c_2 vanishes, leaving c_1 and c_0. The causes, or factors, of variation are still independent and of equal valency, but they do not give results which are as much in excess as they are in defect of the mean. The curve is asymmetrical.

" (3) All three constants, c_2, c_1 and c_0 are left. The contributory factors of variation are not now independent of each other ; they are not of equal valency and they do not give results in excess and defect of the mean with equal frequency. The curve is asymmetrical and may be highly so.

" Therefore many different kinds of curve may be obtained and all of them come from the ' laws of chance.' Some may be symmetrical but we may also have U-shaped and J-shaped curves and even straight lines and triangles—all these cases being included in the fundamental, differential equation. The causes that lead to variability may be numerous, independent and (with respect to the mean) unbiassed—that may be regarded as 'pure chance.' But they may also confer bias, or asymmetry, on the curve; they may be of unequal value in their operation; they may be correlated in groups and they may even be few in number. Thus we may get asymmetrical curves: even J-shaped ones which show the operation of some few, prominent causes of variability as opposed to the multitude of small equal independent causes which result from 'pure chance.' Asymmetry of the curve may go along with homogeneity of the material—but it may also go along with heterogeneity. Thus Pearson and his pupils have shown that an asymmetrical curve may be the resultant of several normal curves and they have even elaborated a method for the analysis of such an asymmetrical curve."

The following distribution gives the lengths and ages of all the plaice caught on a certain fishing-ground (Nelson Buoy) during September, 1920. These were various samples, but it is only accident that this was so. All the fish might have been collected by the same drag with a trawl net. They were taken in several lots for convenience in the troublesome work of reading the otoliths.

Mean length in cms.	Age-group I.	Age-group II.	Age-group III.	Age-group IV.	Totals
17	7	3	...	...	10
19	7	34	1	...	42
21	4	75	10	...	89
23	...	64	23	...	87
25	...	25	36	1	62
27	...	15	45	1	61
29	...	...	10	6	16
31	...	...	8	...	8
33	...	...	7	4	11
35	...	...	1	1	2
37	...	...	...	1	1
Totals	18	216	141	14	389

The graph of the crude data is given in the histogram (Fig. 4). It is asymmetrical, but it would be difficult to deduce from the form of the graph that the material is heterogeneous in respect of age. At any rate a Type III (or Type I) curve will fit the data. We have calculated a Type III curve and find that the "fit" is 0·44. Each of the age-groups I to IV is represented in the figure by a separate curve deduced from the data obtained by classifying the measurements according to the number of rings on the otoliths. Gaussian curves are calculated for groups II and III. The data for year classes I and IV are very few, so that the curves for these groups are drawn free hand. Adding the ordinates for the various groups we get a very satisfactory correspondence with the Type III curve calculated for the "total" column of the table.

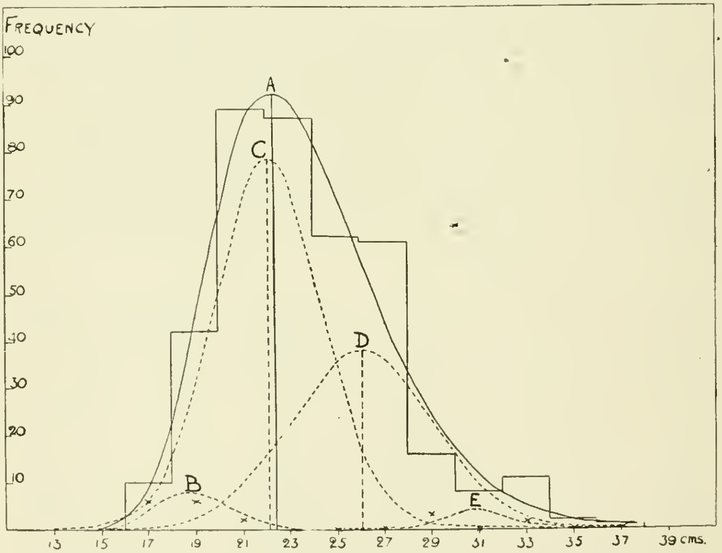

Fig. 4. A catch of plaice from the same ground and month. The histogram represents the crude data; curve *A* shows the graduation from type III; curve *C* is Gaussian and represents group II; curve *D* is also Gaussian and represents group III; curves *B* and *E* are drawn by inspection of the crude data—the crosses in the figure.

Thus we are able to construct a Type III curve from an assemblage of plaice made up of four different age groups. If there is any doubt about the value of the age estimation from otoliths, we might argue that in our sample of plaice the age might be group II, the rest being merely variants. But this method of age estimation by the examination of otoliths in plaice has been verified by experiment and is an actual fact. The practice of age estimation by scale rings has been tested and found correct in the case of the Gadoids and Salmonids. We have applied both methods to herrings and find agreement between scales and otoliths, and we see no reason for rejecting these methods of age estimation for this fish.

How are we going to reconcile these two positions, namely, that we can construct a curve from a sample of herrings which suggests that variations in length and scale rings are due to chance and do not indicate age, and at the same time we can construct a similar type of curve from a sample of plaice in which we do definitely know that the variations in length and otolith rings do indicate four different age groups ?

The Statistical Constants for Fig 4.

CURVE A. 389 plaice from Nelson Buoy taken during September, 1920; 6-in. fish trawl. Type III distribution.

Mean length in cms.	Observed frequency	Calculated frequency	Constants
13	...	imaginary	$N = 389$
15	...	0·016	$d = 0{\cdot}34704$
17	10	8·275	$\mu_2 = 3{\cdot}192114$
19	42	44·820	$\mu_3 = 4{\cdot}129003$
21	89	83·313	$\mu_4 = 36{\cdot}090542$
23	87	90·570	$\beta_1 = 0{\cdot}524153$
25	62	71·350	$\beta_2 = 3{\cdot}541896$
27	61	45·295	$\kappa_2 = 0{\cdot}810014$
29	16	24·625	$\gamma = 1{\cdot}546192$
31	8	11·926	$a = 4{\cdot}288852$
33	11	5·275	$y_0 = 92{\cdot}0173$
35	2	2·170	
37	1	0·910	Mean = 23·694 cms.
39	...	0·310	Mode = 22·400 ,,
41	...	0·110	
	389	388·965	

The equation is

$$y = 92{\cdot}0173 e^{-1{\cdot}546192x}\left(1 + \frac{x}{4{\cdot}288852}\right)^{6{\cdot}631377}$$

CURVE C. Calculation of a Normal Curve from the analysis of age group II of the same sample of plaice.

Mean length in cms.	Observed frequency	Calculated frequency	Constants
13	...	0·015	$N = 216$
15	...	0·428	$d = -0{\cdot}449074$
17	3	5·327	$\mu_2 = 1{\cdot}210369$
19	34	28·998	$c = 2{\cdot}420739$
21	75	69·095	$y_0 = 78{\cdot}3258$
23	64	72·064	
25	25	32·901	Mode = mean = 22·101851
27	15	6·573	The equation is
29	...	0·575	
31	...	0·022	$y = 78{\cdot}3258 e^{-\frac{x^2}{2{\cdot}420739}}$
	216	215·998	

CURVE D. Calculation of a Normal Curve from the analysis of age group III of the same sample of plaice.

Mean length in cms.	Observed frequency	Calculated frequency	Constants
15	...	0·037	$N = 141$
17	...	0·365	$d = 0{\cdot}539007$
19	1	2·25	$\mu_2 = 2{\cdot}221883$
21	10	8·84	$c = 4{\cdot}443765$
23	23	22·15	$y_0 = 37{\cdot}73711$
25	36	35·35	Mode = mean = 26·078014
27	45	35·97	
29	10	23·34	The equation is
31	8	9·66	
33	7	2·64	$y = 37{\cdot}73711e^{\frac{x^2}{4{\cdot}443765}}$
35	1	0·43	
37	...	0·046	
39	...	0·003	
	141	141·081	

Description of the Tables.

Tables I to III are analyses of the different samples from the various localities.

Column 1 is the mean length measured from the tip of the snout to the base of the tail (T.cd.) of each year class.

Column 2 is the extreme range of variation in length (T.cd.) observed for each year class.

Column 3 shows the frequency of each year class.

Column 4 shows the percentage of the whole sample occupied by each year class.

Table I. Welsh Herrings.

Year group	Aberdovey Aberystwith October, 1921				Menai Straits December 22, 1921				Menai Straits January 20, 1922				¼ mile E. of Newquay Cardigan Bay October 2, 1922				Aberdovey Cardigan Bay October 17, 1922				Newquay Cardigan Bay November 1, 1922			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1919	198	184-206	15	8·2	...	...	...	...	...	...	...	...	210	198-229	3	18·7	203	203	1	2·2	200	200	1	3·7
1918	199	180-224	128	69·9	212	193-223	14	23·3	196	176-212	31	64·6	222	202-240	6	37·5	216	194-237	29	64·4	218	200-235	17	63·0
1917	213	181-238	29	15·8	226	215-238	4	6·7	206	190-241	15	31·2	221	210-233	3	18·7	229	207-246	9	20·0	239	234-245	3	11·1
1916	249	241-258	2	1·1	232	222-242	20	33·3	...	...	...	...	228	205-245	4	25·0	240	238-243	2	4·4	250	250	1	3·7
1915	246	244-247	2	1·1	235	228-242	11	18·3	228	228	1	2·1	...	...	...	...	242	242	1	2·2	245	240-252	4	14·8
1914	244	244	1	0·5	236	229-245	9	15·0	240	240	1	2·1	...	...	...	...	248	245-252	2	4·4	...	...	...	...
1913	245	231-260	4	2·2	247	247	1	1·7	...	...	...	...	...	...	...	...	...	...	...	...	250	250	1	3·7
1912	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	250	250	1	2·2	...	...	...	...
1911	247	247	1	0·5	230	230	1	1·7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1910	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1909	255	255	1	0·5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total			183	99·8			60	100·0			48	100·0			16	99·9			45	99·8			27	100·0

Table I—*Continued.*

Year group	Newquay Cardigan Bay November 9, 1922				Newquay Cardigan Bay November 15, 1922				Newquay Cardigan Bay November 22, 1922				Newquay Cardigan Bay November 30, 1922				Newquay Cardigan Bay December 5, 1922				Newquay Cardigan Bay December 8, 1922			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1919	207	202-212	2	6·9	215	215	1	3·6	215	215	1	3·4	215	209-222	4	14·3	...	...	...	...	197	190-204	2	7·4
1918	216	210-224	9	31·0	222	214-230	2	7·1	214	210-218	11	37·9	212	205-223	11	39·3	212	205-219	4	13·3	212	202-230	8	29·6
1917	228	225-233	4	13·8	222	212-233	8	28·6	226	212-242	9	31·0	218	209-235	5	17·9	226	210-242	2	6·7	220	214-236	4	14·8
1916	239	231-241	2	6·9	232	220-242	10	35·7	229	225-234	4	13·8	231	220-240	7	25·0	231	226-239	6	20·0	226	213-235	4	14·8
1915	244	235-254	5	17·2	241	232-246	3	10·7	238	235-245	4	13·8	...	...	...	...	239	220-249	10	33·3	230	221-236	4	14·8
1914	242	237-248	2	6·9	237	234-240	3	10·7	...	...	...	...	250	250	1	3·6	257	249-268	3	10·0	234	230-239	2	7·4
1913	247	243-251	3	10.3	252	252	1	3·6	...	...	...	...	...	...	...	...	257	255-260	2	6·7	245	245	1	3·7
1912	250	250	1	3·4	...	...	...	...	...	...	...	...	...	...	...	...	251	246-256	2	6·7	...	...	...	...
1911	253	253	1	3·4	...	...	...	...	...	...	...	...	...	...	...	...	250	250	1	3·3	243	242-244	2	7·4
Total			29	99·8			28	100·0			29	99·9			28	100·1			30	100·0			27	99·9

Table II. Manx Herrings.

Year group	June 24, 1920				September 9, 1920				September 22, 1920				March 3, 1921				May 12, 1921			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1919	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1918	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	217	211-222	3	6·2
1917	202	184-230	106	55·8	211	196-223	11	18·0	212	207-215	3	1·7	207	203-208	9	19·1	214	203-224	10	20·8
1916	202	184-231	70	36·8	232	213-246	6	9·8	219	206-232	7	4·0	217	206-227	9	19·1	225	214-232	14	29·2
1915	215	198-230	11	5·8	236	214-248	14	23·0	229	216-249	36	20·5	225	214-235	12	25·5	225	217-232	12	25·0
1914	192	192	1	0·5	244	235-251	14	23·0	235	222-249	62	35·2	228	215-238	13	27·7	223	209-232	7	14·6
1913	238	233-243	2	1·0	244	235-252	13	21·3	240	216-248	40	22·7	230	222-237	2	4·3	240	238-241	2	4·2
1912	...	...	...	...	244	239-250	3	4·9	238	225-249	15	8·5	237	233-241	2	4·3	...	...	...	...
1911	...	...	...	...	...	...	...	...	240	232-249	12	6·8	...	...	...	...	...	...	...	...
1910	...	...	...	...	...	...	...	...	246	246	1	0·6	...	...	...	...	...	...	...	...
Total			190	100·0			61	100·0			176	100·0			47	100·0			48	100·0

Table II—*Continued.*

Year group	August 3, 1921				September 21, 1922				September 23, 1922				September 30, 1922			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1919	...	...	...	...	198	188-202	3	9·4	211	211	2	6·7	213	209-216	2	7·1
1918	206	184-225	15	62·5	220	198-245	3	9·4	227	212-255	3	10·0	222	215-227	3	10·7
1917	213	210-228	5	20·8	234	216-247	4	12·5	231	208-240	6	20·0	250	250	1	3·6
1916	...	...	...	...	242	234-249	10	31·2	238	208-250	6	20·0	243	232-255	7	25·0
1915	225	225	1	4·2	245	239-251	7	21·9	247	239-262	4	13·3	245	237-252	13	46·4
1914	222	205-238	2	8·3	249	245-252	2	6·2	246	237-251	5	16·7	244	244	1	3·6
1913	...	...	...	...	245	235-257	3	9·4	248	244-257	4	13·3	...	...	...	...
1912	251	251	1	4·2	...	...	...	...	...	...	...	...	243	243	1	3·6
1911	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1910	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total			24	100·0			32	100·0			30	100·0			28	100·0

Table III. Cumberland and Lancashire Herrings.

Year group	Maryport November, 1921				Maryport November 29, 1922				Morecambe January 14, 1922				Morecambe January 24, 1922				Morecambe December 8, 1922			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1919	...	...	...	...	223	223	1	0·8	...	...	...	...	175	175	1	2·2	212	200-224	2	4·1
1918	...	...	...	...	214	208-224	6	5·0	216	216	1	5·6	183	176-190	9	19·6	198	191-235	13	26·5
1917	226	226	1	12·5	238	215-249	5	4·1	220	217-224	2	11·1	206	192-230	3	6·5	237	230-248	10	20·4
1916	...	...	...	...	241	234-250	20	16·5	212	201-223	2	11·1	232	219-244	6	13·0	234	228-242	9	18·4
1915	239	239	1	12·5	242	229-253	33	27·3	228	200-242	5	27·8	239	215-253	12	26·1	241	231-256	8	16·3
1914	237	237-238	2	25·0	244	226-257	28	23·1	242	240-245	4	22·2	246	233-259	12	26·1	238	231-242	6	12·2
1913	240	235-248	4	50·0	245	241-251	14	11·6	219	199-240	2	11·1	252	249-256	2	4·3	...	...	...	...
1912	...	...	...	...	243	230-250	8	6·6	257	254-260	2	11·1	238	238	1	2·2	...	...	...	...
1911	...	...	...	...	240	235-245	4	3·3	...	...	...	...	...	...	...	...	...	...	...	...
1910	...	...	...	...	244	244-245	2	1·7	...	...	...	...	...	...	...	...	246	246	1	2·0
Total			8	100·0			121	100·0			18	100·0			46	100·0			49	99·9

ON SOME MALIGNANT TUMOURS IN FISHES.

By Jas. Johnstone.

No apology is needed for a description of the conditions mentioned in the following paper. Knowledge of the affections in man called " cancers " is still very slight, and it has become apparent in late years that these diseases are a growing menace to modern civilisation. It is probable that an increase in our knowledge of the causes of " cancer " will come from observation of analogous conditions in the lower animals, and so any records of malignant tumours in fishes are likely to be of ultimate value. I describe here four cases of sarcoma in the hake, cod, turbot and goldfish, which I have had the opportunity of observing during the last year.

(1) *A Fibrosarcoma in the Ling* (*Molva molva*).

A large ling landed at the Barbican in Plymouth was kindly sent to me by Dr. R. S. Clark, of the Marine Biological Association. Fig. 1 represents (with a reduction of one half) the head of this interesting specimen. The tumour is situated on the right-hand side of the head, above and behind the orbit, over which it has grown to such an extent that the pupil is almost covered up. It is about 11 cms. in length, about 5 cms.

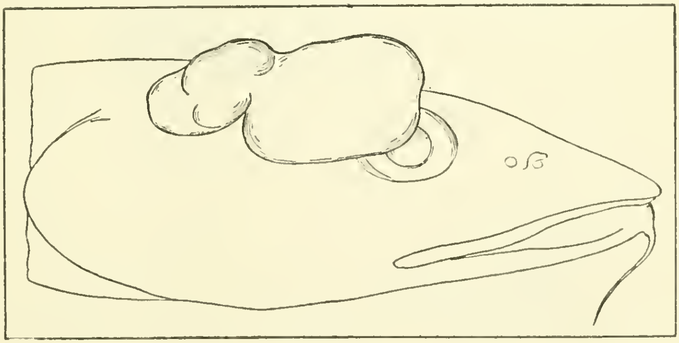

Fig. 1.

in breadth, and it is raised above the general level of the skin by about 3 to 4 cms. It is partially divided into three "lobes" two of which are shown in the outline figure, and the most anterior of these is broken down on the upper surface. There is little doubt that when the ling was alive these tumours were unbroken, but they are always rather soft and the rough handling of the fish during capture and transport had led to rupture of the rather delicate outer investment of the growth. In the interior there is generally much necrotic breakdown of the substance of the tumour.

(2) *A Fibrosarcoma in the Cod* (*Gadus morrhua*).

Fig. 2 illustrates a very similar condition exhibited by a large cod which was observed in the wholesale Fish Market at Liverpool and seized by one of the Inspectors. The tumour, a very obvious and repulsive one, was also situated on the border of the right orbit, between this, the snout and the right-hand upper jaw. It measured about 6 by 3 cms., and had doubtless been raised about 3 cms. above the general level of

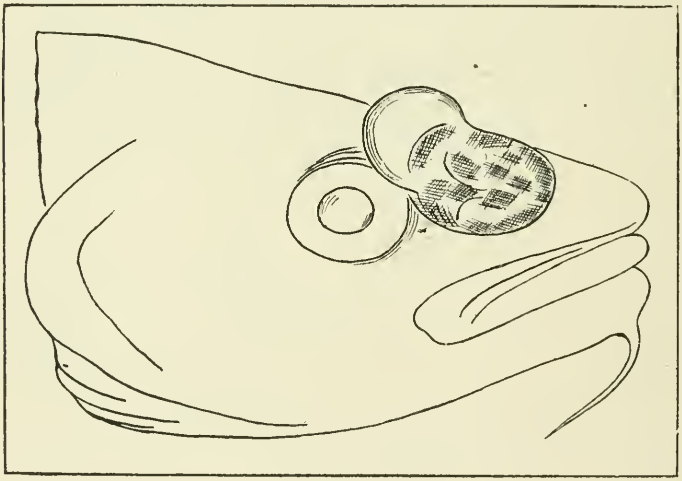

Fig. 2.

the head. There were two parts or "lobes" in the growth. One of these was growing rapidly back over the eye which it had just begun to cover up. The other was growing forward, but the rough handling of the fish had caused it to break down so that an ugly excavated lesion was the result. There had evidently been much necrosis in the central part of the tumour and it had become very soft. The wound was not inflamed or septic.

Sections were made from the marginal parts of the tumours (1) and (2). They are essentially alike and are typical connective tissue overgrowths. In (1) the fibrous tissue predominates, but in (2), and especially in the fully developed parts of the growth, the typical fibrous elements are reduced in mass, and there is an extraordinary development of small round cells. At the margins both tumours are typically malignant. There are no definite boundaries, and the pathological tissue is seen to be intruding itself into the dermis and along the connective tissue septa between the muscle bundles. This condition of infiltration is far better shown in the next specimen to be described, where the conditions are essentially similar. Meanwhile the two fish just mentioned are of much interest in that they afford a welcome confirmation of results already obtained. I have previously described two cod in which the same kinds of sarcomatous tumours were present on the head—in one case the tumour had begun in the region round the orbit and had overgrown the eye and, in the other, it had begun to generalise and had spread into the parts of the head directly beneath the skin and round the upper and lower jaws. We shall see that we are now in a position to generalise somewhat upon the mode of occurrence and growth of sarcomata in marine fishes.

(3) *Fibrosarcoma in a Turbot* (*Rhombus maximus*).

In December of 1922, Mr. T. B. Lovell, collector of statistics for the Ministry of Agriculture and Fisheries in the Brixham area, sent me a large turbot caught in the North Sea, approximately in N. Lat. 50° 26′ and E. Long. 3° 23′ and in water of 19 fathoms in depth. The fish was well grown and nourished, but was remarkable in that it had a very obvious tumour on the coloured side of the body near the root of the tail. The growth was about 8 to 9 cms. in diameter and was raised above the general level of the skin by about 3 to 4 cms. It was round and firm in texture but it had shrunk rather on preservation. On cutting down through it and making a section of the whole body it was seen that the tumour had grown downwards as far as the vertebral skeleton. Its general outline in section was clearly marked, but there was nothing like a capsule round it and the malignant tissue was evidently in process of infiltrating the surrounding muscle substance.

Fig. 3 represents a section made through the marginal zone of the tumour in a plane transverse to the long axis of the body of the fish. On the left hand is shown a part of the

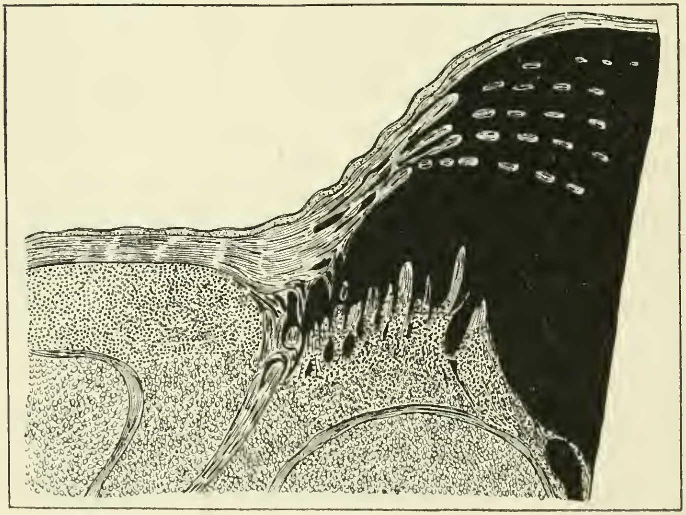

Fig. 3.

normal skin of the fish, with the unaltered muscle substance lying underneath it, and on the right is shown the transitional region between this and the fully developed tumour. The representation of the tissues is conventional. The epidermis is shown by a double line and the upper layer of the dermis by a stippled zone. Beneath this is the coarse, fibrous layer usually found in fishes. There are bundles of white connective tissue running in various directions (but here mainly transverse to the longitudinal diameter of the fish). From this, deep dermal layer sheets of connective tissue of the same histological character take their origin and run down into the body, dividing up the muscles of the latter into the characteristic myotomes, or "flakes." The muscle substance itself is represented by small, roughly circular outlines. Near the surface the fibres are small in cross section but become larger in the deeper parts of the body. Three of the connective tissue septa, or dissepiments, that bound the myotomes are represented in the figure.

In order to show as clearly as possible the relations of the abnormal, to the normal tissue the substance of the tumour is represented in the solid black. It is seen intruding into the underlying muscle substance as noticeable finger-shaped processes. This, however, represents the later growth of the tumour for its first mode of infiltration is along the layer of coarse white connective tissue that is described above as the deep dermis. In Fig. 3 this is wanting altogether in the fully developed tumour, and even in the part of the growing region included in the figure it is seen to become frayed out, so to speak, and to disappear. The intrusion of the malignant growth between the bundles of white connective tissue is shown by the extension of the black area from the right hand.

Fig. 4 represents, in rather more detail, various regions of the tumour and its surroundings. At *A* we have a vertical transverse section of the unaltered skin and subjacent layers.

The epidermis here is not a stratified epithelium but rather a thin layer of round cells which rest on a very sharply marked, "basement membrane." Very characteristic of this layer of the skin in most fishes are the mucous, "goblet" cells, some of which are represented in Figs. 4, A and 4, B. Beneath the basement membrane is an irregular layer of fibrous tissue loaded with pigment and below this again is a much thicker layer which consists of rather fine, white connective tissue fibres

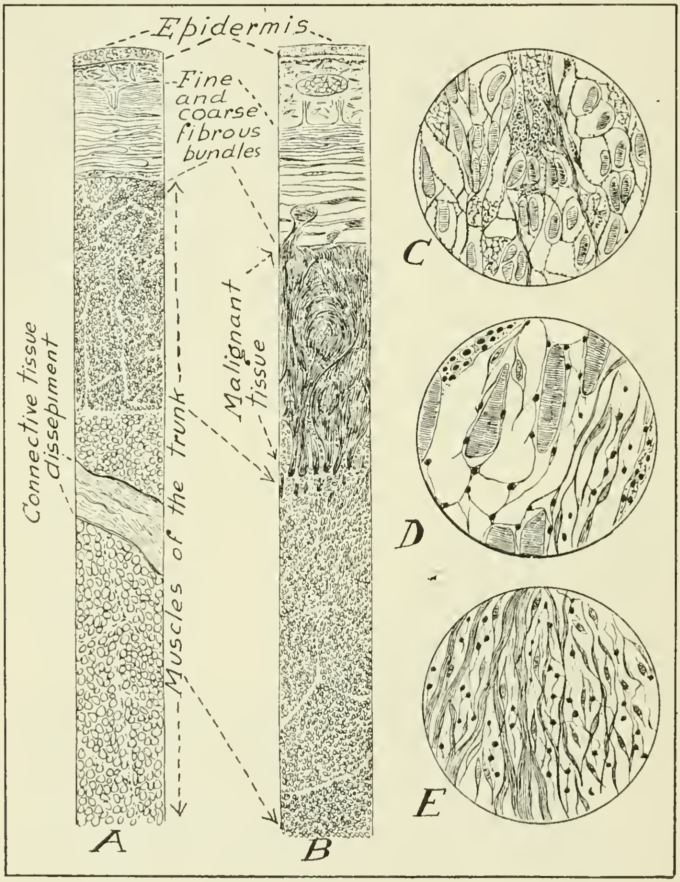
Epidermis
Fine and coarse fibrous bundles
Connective tissue dissepiment
Muscles of the trunk
Malignant tissue
A
B
C
D
E

FIG. 4.

running in various directions. Along with this is areolar tissue.

Below this again is the thick layer of coarse white connective tissue bundles already mentioned. Bundles of connective tissue proceed downwards from the upper fibrous layer of the dermis, traversing the lower coarse layer. From the latter itself, there proceed the strong fibrous sheets of tissue that break up the muscle substance into the myotomes. One of these is shown in Fig. 4, A. Above it there is a region of muscle fibres of large diameter and then the region of small-diameter muscle fibres that lies directly beneath the dermis.

Fig. 4, B, represents a section made through the growing marginal part of the tumour. The malignant tissue is that present in the area shown by fine wavy lines and stippling. It is seen intruding into the layer of coarse connective tissue bundles above, and into the muscle substance below, where the little finger-shaped processes or " roots " of the malignant tumour are again represented. Below the region of the tumour is that of the ordinary large-fibred muscular tissue.

A few words must be said about the nature of the malignant tissue itself. This is represented in Fig. 4, E, where we have evidently a " connective tissue." The elements of the latter are (1) The coarse, white connective tissue bundles similar in all respects to those found in the layers of the dermis, except that they are usually a good deal smaller in diameter. They run in all directions, taking the tumour as a whole, but in any one small field of view most of these fibres will be found to be taking the same course (as in Fig. 4, E). They may intertwine or anastomose. On the whole these are the most obvious elements of the fibrosarcoma as seen in fishes. (2) Small spindle-shaped or fusiform cells of which many are represented in the figure. They have large, pale oval nuclei which are coarsely granular. They appear to be isolated cells in the general complex. (3) A network of fine (?) elastic fibres with small, round cells at its nodes. This occurs everywhere.

A fish sarcoma may be (1) almost entirely fibrous; (2) consisting typically of spindle-shaped or " oat-shaped " cells; or (3) made up almost entirely of small round cells. These are, roughly, the three series of structures just mentioned and any one category may become dominant in the fully developed tumour. As a rule the condition is that all three are present (Fig. 4, E) and that the fibrous elements preponderate. Yet it may be noted that search through any series of sections of a tumour will disclose fields where either of the three forms of malignant tissue may be the principal one present.

Figs. 4, C and D represent small fields in the growing margin: they refer to the same condition but D is the appearance seen under a high magnification. Here the tumour is invading the muscular substance. Now we may look, for a moment, at the normal condition of the latter in a fish. Fig. 5, B, represents several muscle fibres as seen under a high-power lens. Each fibre is invested in its own sarcolema but, in addition to this there is a network of fibres surrounding the fibres, and

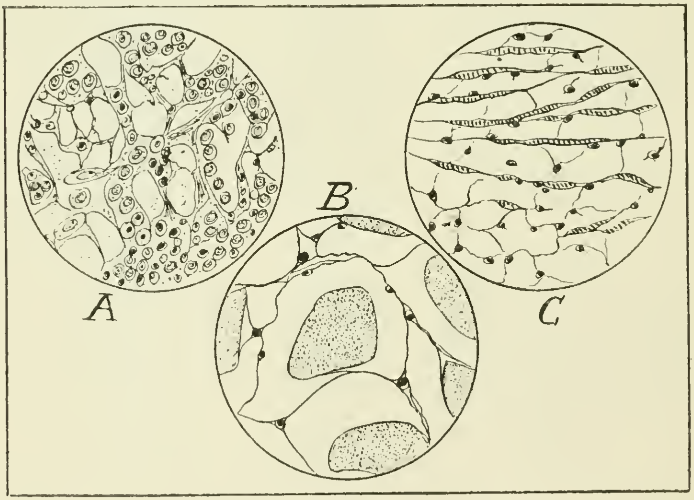

Fig. 5.

these are continuous with the fibrous investments of the muscles, as wholes. In a fish (or at least in the body musculature) there are no very evident muscular units, that is we have what may be regarded as a continuous series of longitudinal muscles extending the entire length of the body. These, however, are broken up into segments, myotomes, or blocks of muscular tissue by the sheets of white, connective tissue fibres referred to in the above paragraphs. These sheets of tissue are continuous with the deep dermal connective tissues, and they are also continuous with the network of fibres which I have just mentioned. The large, approximately transverse dissepiments are both the origins and insertions of the muscles of the body.

Between the individual muscle fibres the connective tissue system, then, takes the form of these networks of fine fibres, with small round cells at their nodes. *It is along this system of fibres that the condition of malignancy evidently extends or grows.* The network of fine fibres itself grows; the small round cells multiply, and a series of relatively coarse, white connective tissue bundles become superadded. The progress of the infiltration of the sarcoma can be seen in Fig. 4, C and D. The network, which in the normal animal serves only as the support for the contractile tissues increases far beyond the degree to which it is useful. At the same time the muscle fibres atrophy. They are really starved because of the growth of the connective tissue networks. The latter increase more and more until the obvious naked eye appearance of a tumour is set up. The connective tissue is continuous everywhere throughout the body and so the way for expansion is provided. Uncontrolled growth of the connective tissues, therefore, proceeds without bounds and unchecked, and to the damage and ultimate death of the contractile tissues. This is the condition of malignancy.

(4) *Fibrosarcoma in a Goldfish* (*Carrasius auratus*).

The specimens described hitherto have always been fish that were killed on capture. In March, 1922, however, a small Goldfish about 8 cms. long, which had an obvious tumour on the right-hand side of the body near the root of the tail was obtained. The fish was kept alive in a small aquarium in the laboratory until the end of June, 1922, when it died. During this time it fed normally except towards the end of June.

Fig. 6 is a nearly full-size drawing of the fish after death. During the three months or so while it was under observation the tumour increased notably in size. The scales over it gradually dropped off. The fish itself did not appear to become emaciated nor were its movements abnormal in any noticeable way.

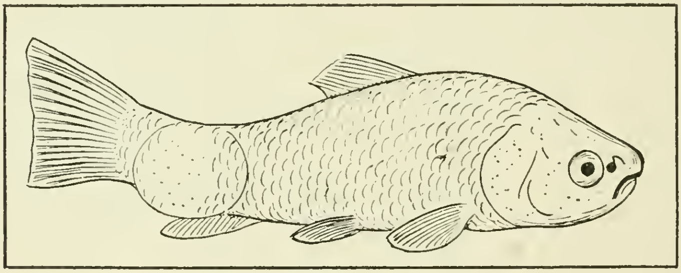

Fig. 6.

On preparing it for sectioning, however, it was seen at once that there was very considerable emaciation. Formalin preservation kept it very plump and apparently well nourished, but this was due to the imbibition of water that always results from this method of preserving a fish. The part of the body containing the tumour was cut out and hardened for sectioning, and during that process the tissues contracted to an exceptional extent. This contraction, it was found was due entirely to a remarkable atrophy, everywhere throughout the body, of the muscle substance.

Fig. 7 represents a transverse section of the part of the body containing the tumour. On the left the latter is represented by the large cross-hatched area, and on the right are the greatly shrunk myotomes (the muscle substance). The drawing is rather conventional inasmuch as it appears to show a definite boundary to the tumour, but in reality, there is no such limit. The growth is not encapsulated in any way, and all round its margin it gradually merges into the surrounding tissues.

Fig. 5 represents three parts of the body seen, in section, under a moderately high magnification. In A the tumour itself is represented, the field being that part of Fig. 7 represented by the coarse stippling towards the outer left-hand side. Here the tissue consists of a very irregular, connective tissue network, which includes large spaces and many groups of cells, mostly small round ones but also some rather larger ones. There are, of course, " overgrown " connective tissue cells, such as those represented in D and E, Fig. 4. B, in Fig. 5, shows a field nearer to the internal boundary of the tumour and here we see just the same arrangement shown in D, Fig. 4 : that is, a growth of the connective tissue between the individual muscle fibres, the small connective tissue cells that are situated mostly at nodal points of this network, and the shrunken muscle fibres (the finely stippled areas in the figure).

But even in the parts of the body remote from the tumour itself this condition of overgrowth of the intermuscular tissue, and the atrophy of the individual muscle fibres, has taken place. Fig. 5, C, illustrates this condition in a part of the body musculature well outside the apparent limits of the tumour. Here the fibres are cut longitudinally and it will be seen that they have shrunk to a remarkable extent. This condition, more or less, is characteristic of the muscle everywhere in the

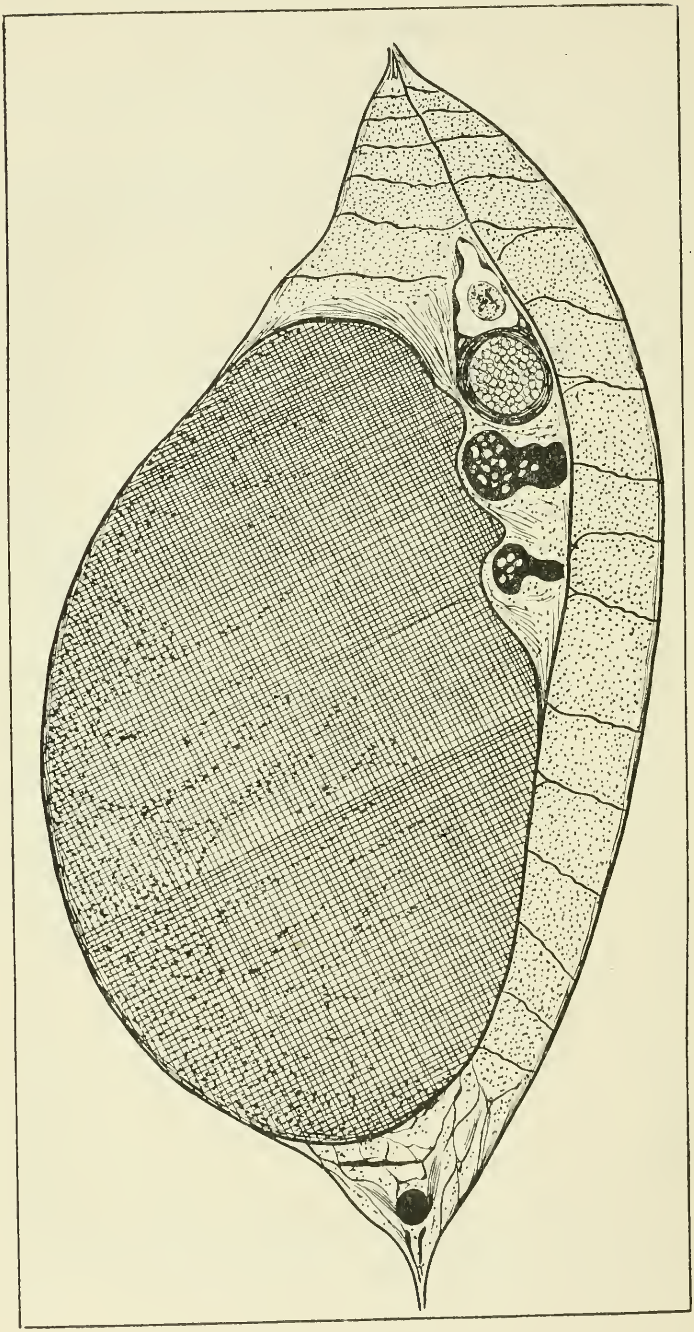

Fig. 7 (Enlarged).

body of the fish. What we have is a local tumour involving the connective tissues in a relatively small part of the body and then a generalisation of this connective tissue overgrowth. In the local growth the muscular tissue has disappeared entirely, while elsewhere it is greatly reduced. Death of the fish has apparently followed from the general lack of nutrition of the organs that are immediately concerned with vital functions, which lack of nutrition is consequent on the uncontrolled overdevelopment of a tissue that has only an incidental part to play in the maintenance of the condition of life.

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